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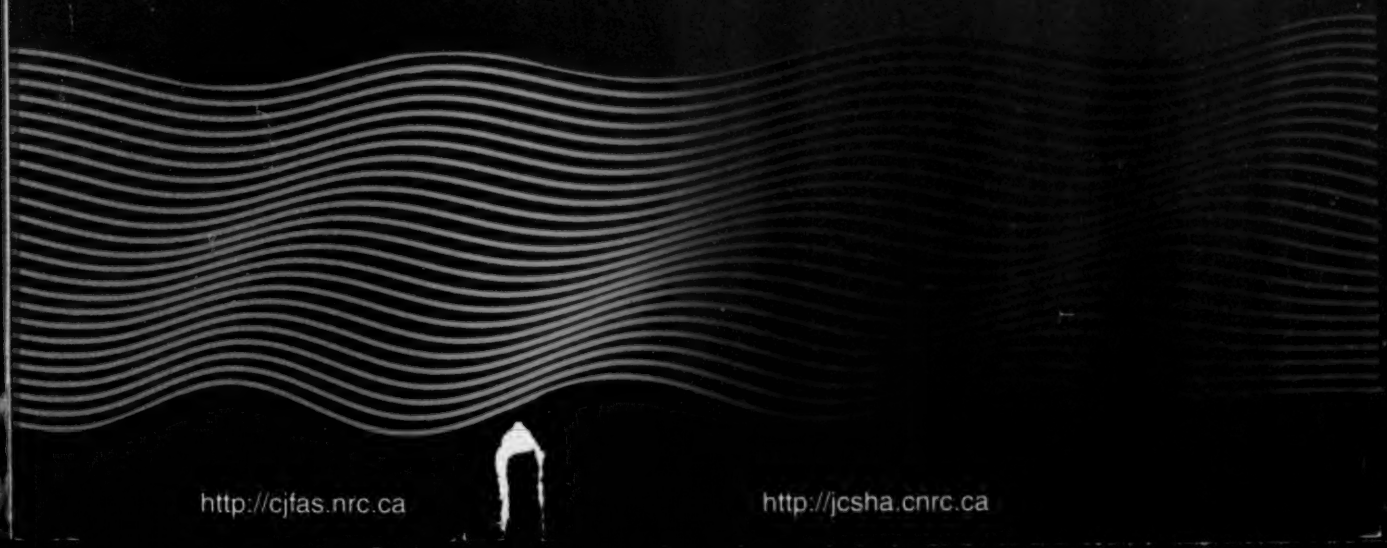

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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Climate change: the potential for latitudinal effects on algal biomass in aquatic ecosystems

Kyla M. Flanagan, Edward McCauley, Frederick Wrona, and Terry Prowse

Abstract: Arctic aquatic systems are considered to be highly susceptible to climate change. Both increases in temperature and nutrient input would be anticipated to alter primary production within these lakes. Consequently, understanding the current relationship between nutrients and productivity is crucial for predicting the effects of climate change. In this paper, we synthesize published data on algal biomass, total phosphorus, total nitrogen, maximum depth, altitude, longitude, and latitude to determine whether average algal biomass differs for temperate and arctic lakes. A total of 57 sources were used, resulting in data for 433 lake-years, ranging in latitudes from 41 to 79°N. Average algal biomass observed during the ice-free season increased significantly with phosphorous levels, but the latitude of the system had a significant negative impact on algal biomass. We briefly outline two major hypotheses, based on existing empirical evidence, for the lower algal yield found in higher latitude systems. The first hypothesis discusses bottom-up control and the influence of abiotic factors on algal biomass. The second hypothesis relates to food chain composition and top-down influences. The latitudinal effect on algal yield suggests that arctic lakes could dramatically increase in productivity if these systems experience increases in temperature and nutrient concentrations as predicted by climate change models.

Résumé : Les écosystèmes aquatiques de l'Arctique sont considérés comme très sensibles aux changements climatiques. Les augmentations tant de température que d'apports de nutriments semblent devoir modifier la production primaire de ces lacs. Il est donc essentiel de comprendre les relations qui existent actuellement entre les nutriments et la productivité si l'on veut prédire les effets des changements climatiques. On trouvera ici une synthèse des données publiées sur la biomasse des algues, le phosphore total, l'azote total, la profondeur maximale, l'altitude, la longitude et la latitude, qui cherche à déterminer si la biomasse moyenne des algues est différente dans les lacs tempérés et les lacs arctiques. Un total de 57 sources dans la littérature ont fourni des données sur 433 lacs-années sur une étendue de latitudes de 41 à 79 °N. La biomasse moyenne des algues mesurée pendant la saison de dégel augmente significativement avec les concentrations de phosphore, mais la latitude du système a un effet négatif significatif sur la biomasse des algues. Nous décrivons brièvement deux hypothèses fondées sur des données empiriques pour expliquer la production réduite d'algues dans les systèmes des latitudes plus élevées. La première se base sur le contrôle ascendant et l'influence des facteurs abiotiques sur la biomasse des algues. La seconde est reliée à la composition de la chaîne trophique et aux effets descendants. L'effet de la latitude sur la production des algues laisse croire que les lacs de l'Arctique pourraient voir leur productivité s'accroître de façon spectaculaire s'ils subissent une augmentation de température ou de concentration de nutriments, comme le prédisent les modèles de changement climatique.

[Traduit par la Rédaction]

Climate models have yielded predictions regarding how temperature and moisture will change in response to different levels of greenhouse gases. Debate exists with respect to how aquatic ecosystems will respond to these changes (Carpenter et al. 1992; Rouse et al. 1997; Hobbie et al. 1999). Water bodies across Canada, differing in geology, food-web structure, energy, and nutrient input will almost certainly respond variably. Therefore, an important step for predicting the effects of climate change on aquatic systems involves identifying regions considered to be most vulnerable to cli-

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K.M. Flanagan and E. McCauley.[1] Ecology Division, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada.
F. Wrona and T. Prowse. Aquatic Ecosystems Impacts Research Branch, National Water Research Institute, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada.

[1]Corresponding author (e-mail: mccauley@ucalgary.ca).

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matic perturbations. Arctic aquatic ecosystems are of particular interest as they are considered to be highly susceptible to structural and functional alterations resulting from changes in climate (Hobbie et al. 1999). Climate models predict a disproportionate increase in temperature for this region, as large as 4–7.5°C in the summer and 2.5–14°C in the winter (IPCC 2001). As well, increased precipitation and permafrost thawing are expected to augment the currently minimal levels of nutrient and carbon input from the terrestrial surroundings (Rouse et al. 1997).

Both the increase in temperature and nutrient input would be anticipated to alter primary production within these lakes. However, developing a mechanistic model to accurately predict the consequences of climate change is not possible, at present, because of limited process-oriented data for arctic lakes across a range of latitudes. In this paper, we draw together empirical observations for lakes across North America, including the Arctic, and show that average algal biomass during the ice-free season is significantly negatively related to the latitude of the system independent of the nutrient concentration. We outline two major hypotheses for the lower algal yield found in higher-latitude systems based on existing empirical evidence. The latitudinal effect on algal yield suggests that arctic lakes could take a big leap in productivity if these systems increase in temperature and nutrient concentration as predicted by climate change models.

We examined the relationship between algal biomass and latitude using data drawn from the literature on algal biomass (chlorophyll *a* (Chl *a*)), total phosphorus (TP), total nitrogen (TN), latitude, longitude, altitude, and maximum depth. A total of 57 separate sources were used (a complete set of data and citations are available),[3] resulting in data for 433 lake-years (269 different lakes). The range of latitudes used in this study was from 41 to 79°N with all lakes located in the Western Hemisphere. Arctic lakes (latitudes ≥60°N) represented ~116 of the lake-years, with the remaining observations from the North Temperate Zone (latitudes <60°N). Although the division of lakes into temperate and arctic based on latitude is artificial, it provides a rudimentary comparison between higher-latitude lakes and lower-latitude lakes; we also considered trends using latitude as a continuous variable to eliminate any biologically irrelevant categorization of lakes. Mean epilimnetic summer values (during the ice-free season) were used to better represent algal levels; however, some point observations were included (5.2%). Examination of the residuals indicated that the point observations do not influence our results.

Using a locally weighted sequential smoothing technique (LOWESS), nutrient concentrations (TP, TN) were examined across the range of latitudes to determine if a latitudinal gradient existed. To assess if the Chl *a* – TP relationship differed between arctic and temperate regions, we used two approaches. First, the lakes were grouped by latitude (arctic ≥60°N; temperate, <60°N), and regression equations were compared by testing for significant differences in slopes and intercepts. Second, multiple regression analysis with variable selection by backward elimination was performed to determine the empirical model that best predicted Chl *a* over the geographic range examined. The multiple regression analysis was performed on the data set containing 433 lake-years, as well as on a modified data set containing averages from lakes sampled in multiple years of replicate lakes to eliminate potential autocorrelation between years. We included higher-order terms (i.e., log(TP), $(\log(TP))^2$) to capture nonlinearities in the relationship. In addition, other independent variables describing the location (latitude, longitude, and altitude) and physical (maximum depth) and chemical (TN) features of the water body were included in the model.

Nutrient levels (TP, TN) were highest in the mid-latitudes (~50–60°N), peaking in the 50–55°N range, then declining after ~60°N, and remaining lower at latitudes above this (Fig. 1). When the data were separated by region into temperate and arctic lakes (Fig. 2*a*), a difference in the response of Chl *a* to increasing phosphorus concentration was observed. Lakes in temperate regions followed the equation $\log(\text{Chl } a) = -0.382 + 0.921\log(\text{TP})$ ($r^2 = 0.28$, $n = 316$, $P < 0.05$). The best Chl *a* – TP relationship for arctic systems was $\log(\text{Chl } a) = -0.418 + 0.332\log(\text{TP})$ ($r^2 = 0.07$, $n = 113$, $P < 0.05$). Although lakes from both regions displayed positive relationships, with considerable variation at a given level of phosphorus, the slopes of these relationships were significantly different ($P < 0.05$; *t* test of regression coefficients). Given that the intercepts were not significantly different ($P > 0.05$), increasing phosphorus levels caused a significantly larger increase in average algal biomass in temperate-zone lakes compared with arctic systems.

To examine how Chl *a* varied over a wide range of latitudes while controlling statistically for other covariates (TP, TN, altitude, longitude, maximum depth), we performed a multiple regression with variable selection by backward elimination. After elimination ($P = 0.05$), the variables remaining in the model were latitude, log(TP), and $(\log(TP))^2$. The relationship governed by the regression equation $\log(\text{Chl } a) = 0.01 + 2.13\log(TP) - 0.38(\log(TP))^2 - 0.03\text{latitude}$ ($r^2 = 0.75$, $n = 433$, $P < 0.05$) is illustrated in Fig. 2*b*. The same trend was produced using the averaged data set, described by the equation $\log(\text{Chl } a) = 0.38 + 1.74\log(TP) - 0.28(\log(TP))^2 - 0.03\text{latitude}$ ($r^2 = 0.70$, $n = 269$, $P < 0.05$). Interestingly, latitude has a negative effect independent of phosphorus level, and other location variables were eliminated in the model selection.

It has previously been shown that arctic lakes have lower primary productivity than temperate lakes (Shortreed and Stockner 1986). Our analysis suggests that for a given increase in phosphorus concentration, the algal biomass of arctic lakes increases less rapidly than lakes in the temperate zone. Consequently, the lower producer biomass is not simply accounted for by lower phosphorous concentrations in the Arctic. Debate exists over whether arctic lakes are typically limited by nitrogen or phosphorous or both (Gregory-Eaves et al. 2000; Levine and Whalen 2001). In this analysis, nitrogen was not a significant predictor of algal biomass, indicating that phosphorous is the primary nutrient limiting

[3] Supplementary material may be purchased from the Depository of Unpublished data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada. DUD 3503. For more information on obtaining material refer to http://www.nrc.ca/cisti/irm/unpub_e.shtml.

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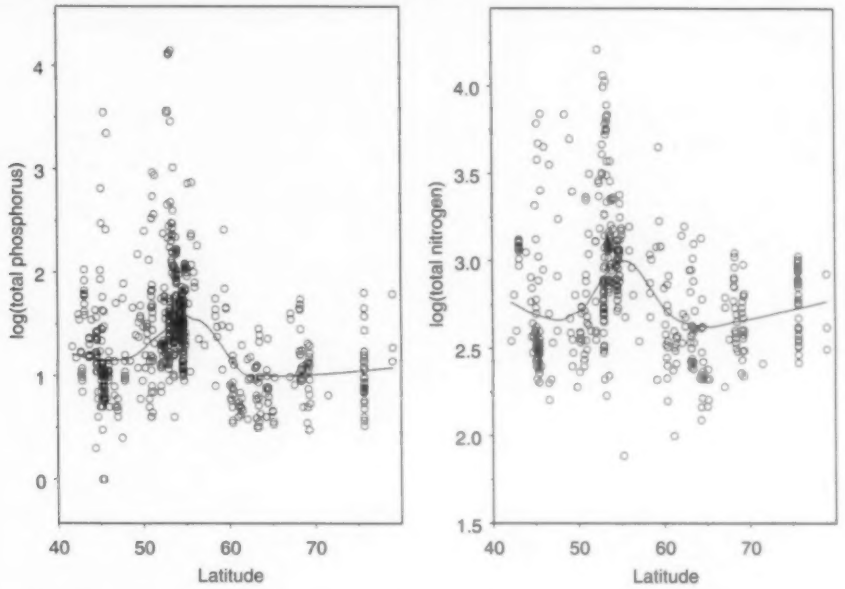
Fig. 1. The observed nonlinear trend in nutrient concentration over the range of latitudes in this study (~40–79°N). Both the total phosphorous and total nitrogen are shown. LOWESS curves are shown on the graph.



algal biomass for these lakes. In addition, the TN:TP ratios for arctic lakes in this sample are not indicative of nitrogen limitation, as determined by comparison with the established TN:TP ratio for nitrogen-limited lakes worldwide (TN:TP < 14) (Downing and McCauley 1992). Arctic lakes in our sample generally had TN:TP ratios greater than 14, which suggests a low probability of nitrogen limitation. Examination of detailed phytoplankton community structure observations from arctic LTER (long-term ecological research) sites indicates that there is no fundamental shift in taxonomic group composition between temperate and arctic phytoplankton communities. This suggests the difference in the Chl *a* – TP relationship between temperate and arctic lakes is not an artifact of changes in the Chl *a* : biomass ratio resulting from a taxonomic shift. Finally, we examined whether residual deviations were independent of spatial distance among lakes by testing whether covariance in residual deviations was related to distance among lakes. There was no detectable effect, suggesting that potential spatial autocorrelation does not influence our conclusion regarding the effect of latitude.

Why is the average level of algal biomass lower in arctic systems than in temperate systems with similar levels of total phosphorus? What characteristics of the arctic environment may be limiting the accumulation or growth of algal biomass? Here we present two hypotheses to explain the discrepancy in the standing stock of algal biomass between the two regions. The first hypothesis relates to bottom-up control on algal biomass; the second hypothesis relates to food chain composition and top-down influences. These are not mutually exclusive and there may be others. We forward these extreme explanations simply to draw attention to differences in mechanisms governing the accumulation of algal biomass in arctic and temperate lakes.

Arctic lakes have a number of abiotic characteristics that distinguish them from temperate lakes. Arctic lakes are typically low energy, having reduced absorbed radiation, high surface albedo, and low temperatures and are subjected to extreme variations in day length (Duff et al. 1999). These lakes are covered with ice and snow for more than 8 months of the year (Hobbie et al. 1999), resulting in a short growing season with most biological activity compressed into a just few months. There are a number of potential mechanisms by which these conditions may limit growth and accumulation of algal biomass. The low temperatures in the Arctic may exacerbate the effect of the existing low nutrient levels by decreasing the activity of enzymes, thus reducing the catalytic power per unit of nutrients invested in the enzyme (Markager et al. 1999). Phytoplankton from high-latitude, cold, freshwater lakes have extremely low photosynthetic rates and low rates of carbon fixed per mole of photons absorbed, thought to be due to a temperature-induced decrease in enzyme activity (Markager et al. 1999) or the reduced availability of cofactors (Gregory-Eaves et al. 2000). Reduced photosynthetic rates of arctic phytoplankton may result in lowered overall growth rates, and therefore, the lower standing stock of algae may be explained by the extended time required to reach equilibrium, in combination with the short growing season in arctic systems.

Biological interactions, including trophic structure and food chain composition, are also known to influence producer biomass through top-down control. Both top-down and bottom-up processes operate simultaneously (Hansson 1992), but the degree to which each process influences producer biomass varies. The well-known model developed by Hairston, Smith, and Slobodkin (HSS, described in Hansson (1992)) describes the relative influence of top-down and bottom-up control in terrestrial ecosystems as a function of system productivity. In

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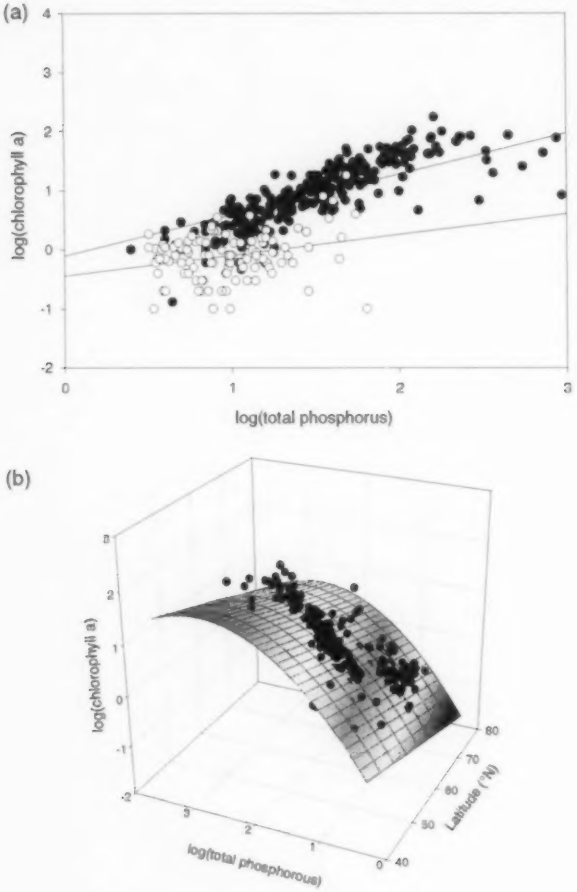
Fig. 2. (*a*) The Chl *a* – TP (chlorophyll *a* – total phosphorus) relationship for temperate and arctic systems. The difference in the slope is statistically significant. Solid circles represent temperate lakes and open circles represent arctic lakes. (*b*) The graphical representation of the model produced by multiple regression to predict Chl *a* depending on log(total phosphorus) and latitude (degree decimals).



this model, very unproductive systems are expected to contain only primary producers, which because of the absence of any top-down control, are completely resource limited (bottom-up dominated). Bottom-up dominated systems are predicted to show a positive relationship between increasing nutrient concentration and producer biomass. As the productivity of the system increases, grazers invade (two-level system), resulting in the producer biomass being grazer regulated and top-down dominated. Top-down dominated systems are predicted to show little or no increase in producer biomass with increasing nutrient levels because grazers are completely resource limited and uninhibited to increase in response to any increase in producer biomass. The resource-limited growth of the grazers results in a relatively constant accumulation of producer biomass across nutrient levels. If the productivity of the system increases further, predators will invade (three-level system) and the primary producers will be released from grazer regulation and once again become resource limited (bottom-up dominated) (Hansson 1992).

Hansson (1992) suggested that the HSS model could explain differences observed in the Chl *a* – TP relationship between two- and three-level aquatic systems. The study compared the Chl *a* – TP relationship of Swedish lakes (known three-level system) to unproductive Antarctic lakes (known two-level system). The slope of the Chl *a* – TP relationship for the Antarctic lakes was significantly less than for the Swedish lakes. This was hypothesized to be a consequence of the different trophic structures of the lakes as productive (three-level) Antarctic lakes showed a Chl *a* – TP relationship similar to that of the Swedish lakes, thus ruling out climatic factors as a source of the difference (Hansson 1992). One possible explanation for the discrepancy in the Chl *a* – TP relationship between the temperate and arctic lakes may be that differences in productivity account for differences in the relationship through trophic level interactions. The low productivity that has been observed in the Arctic may limit the presence of predators (fish) and may result in a system in which algal biomass is controlled by extensive zooplankton grazing.

Empirical evidence from Shortreed and Stockner (1986) support the HSS two-level predictions. In their study, subarctic lakes in the Yukon Territory showed higher levels of zooplankton biomass with respect to phosphorus concentrations than in temperate regions. The high abundance of zooplankton was attributed to the low abundance of planktivorous fish (suggesting a two-level system), and lack of zooplankton predators was thought to lead to the overconsumption of algae. The limited presence of fish could also be attributed to landscape constraints in the Arctic rather than to productivity limitations. Hershey et al. (1999) have shown that geomorphic constraints restrict fish access to arctic lakes, resulting in truncated food webs, which would follow expectations for two-level systems.

The observed difference in the Chl *a* – TP relationship for temperate and arctic lakes may provide insight into the effects of climate change. Arctic lakes, although currently relatively unproductive, could experience a large leap in productivity. Reduction of the length of ice cover may dramatically increase the solar radiation available for algal growth. The reduced period of ice cover and increased absorption of solar radiation will also have dramatic effects on the thermal budget of the lake. If temperature increases as predicted, phytoplankton may no longer experience temperature- and light-induced photosynthetic rate inhibition, and growth rates may become more similar to those of the temperate zone, allowing for a greater accumulation of algae. With respect to the second hypothesis, if algae are currently being heavily grazed because of a lack of zooplankton predators, as the productivity of the system increases, more predators would be expected to invade, releasing phytoplankton from grazer control and allowing for an increase in algal biomass. In addition, the predicted increase in nutrient concentration (owing to permafrost thawing) would augment these changes making the increase in productivity even more drastic. Further investigation into the basis for the difference in the Chl *a* – TP relationship between temperate and arctic systems may provide insight into mechanisms controlling algal biomass, provide direction for

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future management, and allow for more accurate predictions regarding the effect of climate change (Rouse et al. 1997).

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A simulation model for designing groundfish trawl surveys

Jon T. Schnute and Rowan Haigh

Abstract: This paper describes a convenient simulation model, based on the compound binomial-gamma distribution, to assist the planning and design of groundfish trawl surveys. The analysis uses swept-area density measurements from stratified tows to give a simple nonparametric biomass estimate. A parametric simulation model requires only three input parameters for each stratum, which can be estimated initially from past surveys or commercial fishery data. Analytical results provide intuitive algorithms for estimating variances, investigating tow allocation strategies, and exploring potential survey results. Simulations make it possible to compare the estimated biomass with its true value and to assess coverage properties of confidence intervals obtained from bootstraps. Bias correction and acceleration both improve the results, but small samples taken from populations with highly variable densities tend to produce underestimates of available biomass. The simulation framework allows easy adaptation to address broader issues, such as the design of a multispecies survey.

Résumé : On trouvera ici un modèle de simulation commode, basé sur la distribution conjointe binomiale et gamma, pour aider à planifier et monter des inventaires au chalut de poissons de fond. L'analyse utilise des mesures de densité sur la surface échantillonnée tirées de pêches stratifiées pour produire une estimation simple non paramétrique de la biomasse. Un modèle de simulation paramétrique nécessite l'inclusion de seulement trois paramètres pour chaque strate, qui peuvent être estimés au départ à partir d'inventaires passés ou de données de la pêche commerciale. Les résultats de l'analyse fournissent des algorithmes intuitifs pour estimer les variances, pour évaluer les stratégies d'allocation des pêches et pour explorer les résultats potentiels de l'inventaire. Des simulations permettent de comparer la biomasse estimée à sa valeur réelle et d'évaluer les caractéristiques de couverture des intervalles de confiances obtenus par bootstrap. La correction d'erreurs et l'accélération améliorent les résultats, mais de petits échantillons tirés de populations à densité très variable ont tendance à sous-estimer la biomasse disponible. Le cadre de la simulation permet facilement des adaptations pour confronter des problèmes plus vastes, tels que la planification d'un inventaire impliquant plusieurs espèces.

[Traduit par la Rédaction]

Introduction

Groundfish trawl surveys commonly use measurements from numerous tows to calculate an overall biomass index. The high cost of vessel time makes surveys expensive, with a significant incremental cost for each additional tow. Furthermore, surveys generally produce highly uncertain results due to variable catch rates among tows. Like market investments, surveys require allocation of research budgets in pursuit of an uncertain return. A suitable planning model needs to contemplate the variability of tow data and investigate its implications for calculating a biomass index with associated uncertainty. In this paper, we formulate such a model, based on historical methods of survey analysis. We then derive analytical results that provide intuitive algorithms for exploring potential survey outcomes, given parameter estimates that describe population density and variability. Simulations allow us to compare the biomass index estimated from survey data with the true biomass index and to assess the coverage properties of confidence intervals obtained by bootstraps or other statistical methods. In particular, we identify circumstances that can lead to deceptive results.

Researchers have proposed various methods for the design and analysis of abundance surveys. Pennington (1983, 1986) examined the use of a compound binomial-lognormal distribution, earlier called a "Δ-distribution" (Aitchison 1955; Aitchison and Brown 1957). This model allows for tows that sometimes produce no catch of the target species, but it requires somewhat cumbersome numerical corrections for known properties of the lognormal distribution. Smith (1988, 1990) investigated this approach further and distinguished such model-based approaches from the design-based methods of finite population sampling. Myers and Pepin (1990) compared these two approaches and found the ordinary sample mean and variance estimates more robust than those derived from the Δ-distribution. They also examined other distributions, including the Weibull and gamma, as models of catch rates among tows that produce nonzero catch. Steffánsson (1996) observed that the gamma distribution belongs to the family of generalized linear models (GLMs) discussed by McCullagh and Nelder (1989). Consequently, a compound binomial-gamma distribution conveniently links the discrete ("Δ-distribution") properties of the binomial with GLM theory.

The analyses described so far focus primarily on mean and variance estimates of biomass. A normal approximation gives confidence intervals symmetric about the mean. Smith (1997) examined the use of bootstraps to obtain more complete descrip-

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J.T. Schnute[1] and R. Haigh. Fisheries and Oceans Canada, Science Branch, Pacific Region, Pacific Biological Station, Nanaimo, BC V9T 6N7, Canada.

[1]Corresponding author (e-mail: schnutej@pac.dfo-mpo.gc.ca).

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tions of uncertainty, including confidence intervals that may not be symmetric around the mean. For simple random samples, Efron and Tibshirani (1993, p. 184–188) demonstrated that interval estimates from a bootstrap distribution could be improved using bias correction and acceleration (BC_a). Smith (1997) presented the modifications of their algorithm required for a stratified random sample from a finite population and cited further refinements proposed by Rao and Wu (1988) and Sitter (1992).

In this paper, we take advantage of these historical developments to formulate a convenient simulation model for planning groundfish trawl surveys. A typical survey consists of numerous tows along the sea floor, where each tow gives a biomass density estimate

$$\text{density} = \frac{\text{biomass captured}}{\text{area swept by the net}}. \tag{1}$$

Total stock biomass can then be estimated from the available habitat area as

$$\text{biomass} = \text{density} \times \text{habitat area}. \tag{2}$$

Surveys usually divide the available habitat into strata, based on criteria known to influence fish density. For example, a survey might be stratified by depth of the sea floor, where appropriate depth strata depend on the species of interest. The estimate (2) then involves a sum over various strata.

If z denotes a random density measurement (1) obtained from a tow, the statistical distribution of z generally requires at least three parameters. Because tows sometimes produce no catch of the target species, measurements $z = 0$ have an associated probability p. Furthermore, the distribution of positive measurements $z > 0$ has a mean μ and coefficient of variation ρ. Our simulation model uses this minimal set of three parameters (p, μ, ρ) for each stratum and species. Historical literature suggests various options for simulating positive measurements, including the lognormal and Weibull distributions (e.g., Myers and Pepin 1990), but we adopt the gamma distribution for its relatively simple analytical properties. As Steffánsson (1996) shows, this often provides a reasonably adequate description of actual tow data. We have explored catch rate patterns in observer trawl data for numerous Pacific groundfish and found that nonzero tow density measurements for target species fit the gamma distribution reasonably well. By contrast, species actively avoided sometimes show density patterns closer to a lognormal distribution. We do not explore this issue further here, but the main results of our simulation exercise are reasonably robust to the distribution chosen for $z > 0$.

Our formulation of the gamma distribution uses the parameter

$$\nu = \frac{1}{\rho^2}, \tag{3}$$

inversely related to the coefficient of variation ρ for positive measurements. Thus, the compound binomial-gamma has parameters (p, μ, ν) equivalent to (p, μ, ρ). We show that the maximum likelihood estimate for the mean agrees with the sample mean. Consequently, our simulation framework integrates model-based estimates with sample-based estimates, which are the same from either point of view. We consider a stratified design and, for a given research budget, calculate the optimal number of tows in each stratum. The triplet of model parameters for each stratum and species can readily be estimated from past surveys, commercial fishery data, or other sources. Analytical formulas make it easy to explore the consequences of parameter changes in space and time.

Investigators have often used finite population sampling theory to design a survey and analyze the resulting data. For example, suppose that a stratum with area A contains $M = A/a$ grid blocks, each with area a. If n blocks are chosen randomly without replacement from the available population of M blocks, then one tow in each selected block gives density measurements z_i $(i = 1, \ldots, n)$. According to the theory (Thompson 1992, p. 15), the variance of the sample mean $\bar{z} = \sum_{i=1}^{n} z_i/n$ can be estimated as

$$\widehat{\mathrm{V}}[\bar{z}] = \left(\frac{M-n}{M}\right)\frac{s^2}{n}, \tag{4}$$

where $s^2 = \sum_{i=1}^{n}(z_i - \bar{z})^2/(n-1)$ denotes the sample variance. The factor $(M-n)/M$ in (4), called a finite population correction (FPC), reduces to 0 when $n = M$. Thus, if all M sampling units are measured, the sample mean is exactly the population mean measured without error (variance 0). This model assumes a fixed measurement z_i for each sampling unit i, as in the case that z_i denotes fish length in a population of M fish.

Christman and Pontius (2000) illustrate this approach with simulations from spatially clustered populations, where each block has a prescribed density. They examine bootstrap methods for an adaptive cluster sampling design. Although their approach has obvious relevance to surveys of spatially stable populations, we regard the model as inappropriate for groundfish trawl surveys. Repeated tows taken from the same grid block of the ocean floor almost never give the same fish density. Thus, the hypothetical density measurement z_i in block i is not well defined. Some highly mobile groundfish species can readily move among blocks during the course of a survey.

Our simulation model addresses this problem with the simple assumption that individual tows represent independent samples from an underlying parent distribution for each stratum. We make no explicit assumptions about how this independence is achieved, although we recognize potential problems when samples are obtained without regard for independence and stratum coverage. For example, data collected entirely from one location in the stratum would obviously not fit the assumptions here. A strict application of sampling design principles can be difficult in groundfish surveys. If tows theoretically represent randomly selected blocks, fishing constraints may prevent a particular tow from covering the designated block or may even force it into adjacent blocks. Sampling design principles can help to achieve independence and coverage, but the model here starts with the assumption that the data somehow meet these criteria. Our BC_a corrections to the bootstrap (Appendix A) reflect this point of view and differ slightly from those presented by Smith (1997) because our model has no finite population correction factor.

For clarity, we compile various model equations into tables, where the letter "T" distinguishes tabular equations from those in the text. For example, (T2.3) denotes the third equation in Table 2. The paper begins with a formal description of the biomass

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calculations (1)–(2). We then derive properties of the binomial-gamma distribution that allow us to embed this model into the biomass analysis. Worked examples use simulation and estimation to test rigorously the coverage properties of empirical and BC_a confidence intervals obtained from bootstraps. Bias correction and acceleration both improve the results, but small samples taken from populations with highly variable densities tend to produce underestimates of available biomass. In particular, the upper confidence bound often has a value too low to cover the true biomass value properly. These results illustrate the value of a simulation framework for planning surveys that span multiple strata and potentially target numerous species. Following Starfield and Bleloch (1986), we employ the model as an intellectual tool for anticipating results from costly field programs.

Biomass estimates

The calculations (1) and (2) gloss over many difficulties. A two-dimensional definition of density fails to account for the fact that fish live in a three-dimensional world. Obviously, (1) applies only to fish that live primarily on the bottom. At best, the method can estimate the fraction of biomass available within the vertical capture zone of the net. Furthermore, area measured with respect to the ocean surface does not accurately represent the true area along a sloping stretch of sea floor. Typically, detailed data on the shape of the sea floor are unavailable, and the density (1) is measured relative to the surface.

Other problems relate to animal behaviour, characteristics of the gear, and substrate type. The effective area swept by the net may be uncertain, particularly due to herding or scattering effects on the fish (Bublitz 1996). Density sampling performs optimally when the fish have a relatively even distribution throughout the habitat. Schooling can cause highly variable estimates in which tows produce frequent low catches interspersed with occasional high catches. Temporal shifts in abundance add another source of variation, although a survey usually occurs during a relatively short time period of interest to the investigator. Furthermore, the habitat area in (2) might be poorly defined, and different habitats might support different densities of fish. Thoughtful stratification schemes could potentially address some of these problems.

Our analysis takes the data at face value and follows the concepts (1) and (2) to their logical conclusion. We assume (Table 1) that the survey covers m strata with n_h tows in stratum h ($h = 1, \ldots, m$). Each tow i in stratum h has a known catch C_{hi}, effort (i.e., tow duration) E_{hi}, vessel speed v_{hi}, and net width w_{hi}. By the logic of (1), the tow produces a density measurement

$$(5) \qquad z_{hi} = \frac{C_{hi}}{v_{hi} w_{hi} E_{hi}} = \frac{1}{v_{hi} w_{hi}} U_{hi},$$

where $U_{hi} = C_{hi}/E_{hi}$ is the catch per unit effort (CPUE). The swept area in (5) is the net width times the distance travelled, where the latter can be expressed as tow duration times vessel speed. The n_h tows in stratum h give the mean density estimate

$$(6) \qquad \hat{\delta}_h = \bar{z}_h = \frac{1}{n_h} \sum_{i=1}^{n_h} z_{hi}.$$

Table 1. Notation for quantities discussed in this paper.

Indices	
h	stratum ($h = 1, \ldots, m$)
i	tow ($i = 1, \ldots, n_h$)
j	confidence quantile ($j = 0, \ldots, Q$)
r	bootstrap replicate ($r = 1, \ldots, R$)
s	simulation ($s = 1, \ldots, S$)
Stratum and survey parameters	
A_h	habitat area in stratum h
A	total area $A = \sum_{h=1}^{m} A_h$
B_h	biomass in stratum h
B	total biomass $B = \sum_{h=1}^{m} B_h$
k_h	cost of one tow in stratum h
n_h	number of tows in stratum h
K	total cost $K = \sum_{h=1}^{m} n_h k_h$
N	total number of tows $N = \sum_{h=1}^{m} n_h$
δ_h	biomass density B_h/A_h in stratum h
Δ	overall biomass density $\Delta = B/A$
Tow data	
C_{hi}	catch biomass
E_{hi}	effort (duration of tow)
U_{hi}	catch per unit effort (CPUE) $U_{hi} = C_{hi}/E_{hi}$
v_{hi}	vessel speed
w_{hi}	net width
z_{hi}	biomass density $z_{hi} = U_{hi}/(v_{hi} w_{hi})$
Binomial-gamma parameters	
p	proportion of zero measurements
μ	mean of nonzero measurements
ρ	coefficient of variation (CV) of nonzero measurements
ν	squared reciprocal CV of nonzero measurements ($\nu = 1/\rho^2$)
δ	distribution mean
σ^2	distribution variance
γ	distribution CV
Bootstrap confidence intervals	
α_j	quantile confidence level $\alpha_j = j/Q$
$\hat{B}^*_{rs}$	r^{th} bootstrapped biomass estimate from data set s
$B'_{j,s}$	upper α_j confidence limit for B from simulation s

If stratum h has area A_h, then (2) gives the estimate

$$(7) \qquad \hat{B} = \sum_{h=1}^{n} A_h \hat{\delta}_h$$

of the total biomass B. We want to investigate properties of this estimate, given a suitable model for the density measurements z_{hi} in each stratum h.

Strictly speaking, we should refer to B as the biomass available to the gear. Depending on herding or scattering properties of the net and other factors, this may be larger or smaller than the actual stock biomass. Investigators usually deal with this problem by treating the estimate $\hat{B}$ as an index, rather than an absolute measure, of biomass. In the model proposed here, each stratum h has a theoretical mean density δ_h available to the gear, and the quantity

$$(8) \qquad B = \sum_{h=1}^{n} A_h \delta_h$$

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Table 2. Properties of $z \sim G(\mu, \nu)$, where G denotes the gamma distribution and ℓ is the negative log-likelihood for a sample of n independent variates $\{z_i\}_{i=1}^n$.

(T2.1) $$g(z|\mu,\nu) = \frac{1}{z\Gamma(\nu)}\left(\frac{\nu z}{\mu}\right)^{\nu}\exp\left(-\frac{\nu z}{\mu}\right)$$

(T2.2) $$\mathrm{E}[z] = \mu$$

(T2.3) $$\mathrm{V}[z] = \frac{\mu^2}{\nu}$$

(T2.4) $$\rho = \mathrm{CV}[z] = \sqrt{\frac{1}{\nu}}$$

(T2.5) $$\ell(\mu,\nu) = n\left[\log\Gamma(\nu) + \nu(\log\mu - \log\nu) + \frac{\nu\bar{z}}{\mu} + (1-\nu)\overline{\log z}\right]$$

(T2.6) $$\hat{\mu} = \bar{z} = \frac{1}{n}\sum_{i=1}^{n} z_i$$

(T2.7) $$\hat{\nu} \text{ minimizes } \ell(\hat{\mu},\nu) = n\left[\log\Gamma(\nu) + \nu(1 + \log\bar{z} - \log\nu) + (1-\nu)\overline{\log z}\right]$$

(T2.8) $$\breve{\nu} = \frac{\breve{\mathrm{E}}[z]^2}{\breve{\mathrm{V}}[z]} = \frac{(n-1)\bar{z}^2}{\sum_{i=1}^{n}(z_i - \bar{z})^2}$$

defines the true biomass index determined by model parameters. With this understanding, we sometimes refer to the biomass, rather than a biomass index. In our simulations, we compare the estimate (7) with the true value (8).

Binomial-gamma distribution

As discussed in the introduction, we use the compound binomial-gamma distribution with parameters (p, μ, ν) to simulate densities z obtained from trawl tows. We summarize (Table 2) important properties of the gamma distribution $G(\mu, \nu)$. The parameter μ represents the mean, and ν determines the coefficient of variation ρ, as in (3) and (T2.4). Our notation follows that of McCullagh and Nelder (1989, p. 287), although other writers (e.g., Gelman et al. 1995, p. 474) use $\alpha = \nu$ and $\beta = \nu/\mu$. S-Plus gamma distribution functions (MathSoft 1999) include parameters called the "shape" and "rate", which correspond respectively to α and β. Steffánsson (1996) uses $r = \nu$ and $\beta = \mu/\nu$, so that his β is the reciprocal of that in Gelman et al. (1995). Readers should exercise caution when comparing results from different references.

The gamma distribution $G(\mu, \nu)$ has a corresponding density function $g(z|\mu, \nu)$ given by (T2.1), where

$$\Gamma(\nu) = \int_0^\infty x^{\nu-1}e^{-x}\mathrm{d}x$$

denotes the gamma function evaluated at ν. This is related to the factorial function: $(\nu - 1)! = \Gamma(\nu)$. The expected value, variance, and coefficient of variation can be expressed analytically in terms of the parameters (μ, ν), as shown in (T2.2)–(T2.4). The function $\ell(\mu, \nu)$ in (T2.5) is the negative log-likelihood function for a sample $\{z_i\}_{i=1}^n$ of n independent variates. It depends on the data via two sufficient statistics

(9) $$\bar{z} = \frac{1}{n}\sum_{i=1}^{n} z_i\,, \quad \overline{\log z} = \frac{1}{n}\sum_{i=1}^{n}\log z_i = \log\tilde{z},$$

where $\tilde{z} = \left(\prod_{i=1}^n z_i\right)^{1/n}$ denotes the geometric mean of the observations. Maximum likelihood estimates $\hat{\mu}$ and $\hat{\nu}$ correspond to the sample mean $\bar{z}$ in (T2.6) and the minimum of the function $\ell(\hat{\mu}, \nu)$ in (T2.7). In particular, $\ell(\hat{\mu}, \nu)$ depends only on ν after $\hat{\mu}$ in (T2.6) is substituted for μ in (T2.5).

The results (T2.2)–(T2.3) suggest another estimate (T2.8) for ν, based on the usual sample estimates for the mean $\mathrm{E}[z]$ and variance $\mathrm{V}[z]$. We use the breve accent ($\breve{}$) to distinguish a moment estimate from a maximum likelihood estimate ($\hat{}$). For the mean, $\hat{\mu} = \bar{z} = \breve{\mathrm{E}}[z] = \breve{\mu}$. By contrast, the parameter ν has two distinct estimates: the minimization (T2.7) determines $\hat{\nu}$, and (T2.8) expresses $\breve{\nu}$ in terms of the sample variance $\breve{\mathrm{V}}[z] = \sum_{i=1}^n (z_i - \bar{z})^2/(n-1)$. Simulations from the gamma distribution confirm the theoretical prediction that $\hat{\nu}$ has lower variance than $\breve{\nu}$, where both estimators show some bias. The simple formula (T2.8) makes $\breve{\nu}$ a convenient initial estimate for the minimization (T2.7).

We summarize (Table 3) properties of the compound binomial-gamma distribution $H(p, \mu, \nu)$, where p is the probability that $z = 0$ and (μ, ν) are gamma parameters for the distribution of $z > 0$. The density function $h(z|p, \mu, \nu)$ in (T3.1) has two components, with probabilities p and $1 - p$. Once again, the parameters determine explicit formulas (T3.2)–(T3.4) for the expected value, variance, and coefficient of variation (Appendix B). In particular, the mean density δ in (T3.2) is the total expected return from zero and nonzero tows: $\delta = \mathrm{E}[z] = p \cdot 0 + (1-p)\mu = (1-p)\mu$.

The negative log-likelihood in (T3.5) depends on three sufficient statistics

(10) $$n_0, \quad \bar{z}_+ = \frac{1}{n - n_0}\sum_{z_i > 0} z_i,$$

$$\overline{\log z}_+ = \frac{1}{n - n_0}\sum_{z_i > 0}\log z_i = \log\tilde{z}_+,$$

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Table 3. Properties of $z \sim H(p, \mu, \nu)$, where H denotes the compound binomial-gamma distribution and ℓ is the negative log-likelihood for a sample of n independent variates $\{z_i\}_{i=1}^n$ with n_0 values $z_i = 0$ and $n - n_0$ values $z_i > 0$.

(T3.1) $$h(z|p, \mu, \nu) = \begin{cases} p, & \text{if } z = 0 \\ (1-p)g(z|\mu, \nu), & \text{if } z > 0 \end{cases}$$

(T3.2) $$\delta = \mathrm{E}[z] = (1-p)\mu$$

(T3.3) $$\sigma^2 = \mathrm{V}[z] = (1-p)(1+\nu p)\frac{\mu^2}{\nu}$$

(T3.4) $$\gamma = \mathrm{CV}[z] = \frac{\sigma}{\delta} = \sqrt{\frac{1+\nu p}{\nu(1-p)}} = \sqrt{\frac{\rho^2 + p}{1-p}}$$

(T3.5) $$\ell(p, \mu, \nu) = (n-n_0)\left[\log\Gamma(\nu) + \nu(\log\mu - \log\nu) + \frac{\nu\bar{z}_+}{\mu} + (1-\nu)\overline{\log z}_+\right] - n_0 \log p - (n-n_0)\log(1-p)$$

(T3.6) $$\hat{p} = \frac{n_0}{n}$$

(T3.7) $$\hat{\mu} = \bar{z}_+$$

(T3.8) $$\hat{\nu} \text{ minimizes } \ell(\hat{p}, \hat{\mu}, \nu)$$

(T3.9) $$\hat{\delta} = (1-\hat{p})\hat{\mu} = \bar{z}$$

where n_0 is the number of observations with $z_i = 0$ and the subscript "+" indicates averages of observations with $z_i > 0$. Thus, $\bar{z}_+$ denotes the geometric mean of positive observations. The maximum likelihood estimate $\hat{p}$ in (T3.6) is simply the proportion of observed zero values, and the estimates $(\hat{\mu}, \hat{\nu})$ in (T3.7)–(T3.8) come from the gamma distribution for positive values. A simple calculation from (T3.2) and (T3.6)–(T3.7) shows that maximum likelihood estimate $\hat{\delta}$ in (T3.9) for $\mathrm{E}[z]$ is

$$\widehat{\mathrm{E}[z]} = (1-\hat{p})\hat{\mu} = \left(1 - \frac{n_0}{n}\right)\frac{1}{n-n_0}\sum_{z_i>0} z_i = \frac{1}{n}\sum_{i=1}^{n} z_i = \bar{z},$$

where adding values $z_i = 0$ to the sum does not change its value.

In summary, the binomial-gamma distribution shares a useful property with the gamma distribution. The maximum likelihood estimate of the expected value is the sample mean, as in (T2.6) and (T3.9). The lognormal and binomial-lognormal distributions do not have this property, a limitation that introduces extra complexity into an analysis based on those models. The biomass estimate (7) does not require any particular assumption about the distribution of densities within a stratum. Yet it is also the natural estimator when tow densities come from a binomial-gamma distribution. Furthermore, analytical expressions for the mean δ, variance σ^2, and coefficient of variation γ provide simple intuitive calculations for survey design and analysis.

Equations (3) and (T2.4) show an equivalence between the parameters ν and ρ. Following McCullagh and Nelder (1989), we have adopted ν as a parameter of the gamma distribution. Because the coefficient of variation ρ has simpler intuitive meaning, we often prefer to use (p, μ, ρ) as the model's primary parameters. For example, (T3.4) shows that the CV γ for all density measurements depends only on the proportion p of zero measurements and the CV ρ for nonzero measurements. (The mean μ of nonzero measurements cancels in the calculation of γ.) Notice from (T3.4) that $\gamma = \rho$ when $p = 0$ and that γ increases as p and ρ increase (Fig. 1*a*).

Sampling design

We summarize (Table 4) analytical results from a survey with binomial-gamma parameters (p_h, μ_h, ν_h), area A_h, and n_h tows at a cost k_h per tow in each stratum h. Formulas (T4.1)–(T4.3) express the stratum mean density δ_h, variance σ_h^2, and total biomass B in terms of these quantities, where (T4.3) echoes our definition of B in (8). Following the logic of Table 3, we then obtain the estimates $\hat{\delta}_h$ and $\hat{B}$ in (T4.4)–(T4.5). These imply the expected value and variance of $\hat{B}$ reported in (T4.6)–(T4.7). The sum (T4.8) gives the total cost K of a survey with n_h tows in stratum h.

For a fixed budget K, we define an optimal survey as one that allocates tow numbers n_h among the strata to minimize the variance $\mathrm{V}[B]$ in (T4.7). Equations (T4.9)–(T4.11) solve the optimality problem (Appendix B), consistent with classical Neyman allocation (Cochran 1977, p. 99) applied to the parametric model here. The quantity X depends only on the input parameters $(p_h, \mu_h, \nu_h, A_h, k_h)$. The optimal design n_h^* allocates tows proportional to the factor $A_h\sigma_h/\sqrt{k_h}$, thus giving highest priority to strata with large area A_h, high density variability σ_h, and low cost per tow k_h. The minimum achievable variance V^* is given by (T4.11), although the analysis so far

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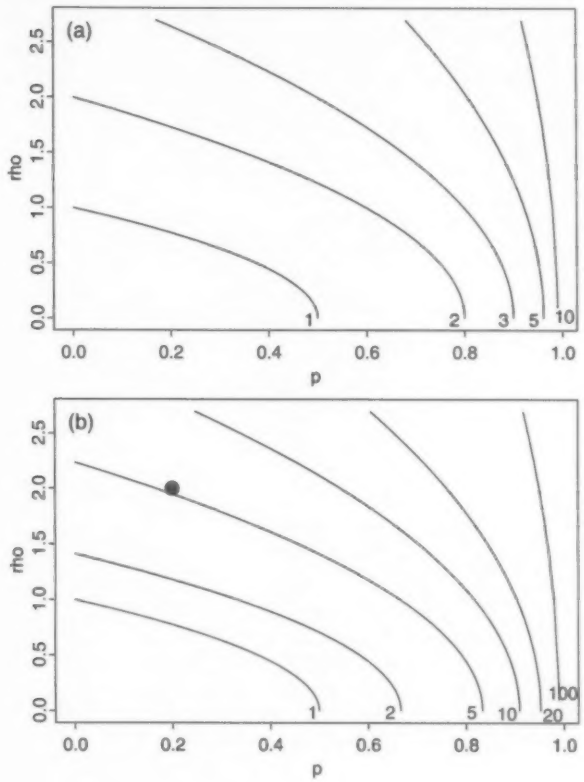
Fig. 1. (*a*) Contours of the coefficient of variation γ in (T3.4) for a binomial-gamma variate with parameters (p, ρ), where γ does not depend on the third parameter μ. (*b*) Sample size n^* required to achieve a target coefficient of variation $CV^* = 1$ in the biomass estimate $\hat{B}$ from a single stratum ($m = 1$). More generally, adjustment by the factor $(CV^*)^{-2}$ gives the sample size needed to meet any desired target CV^*. The circle indicates the point $(p, \rho) = (0.2, 2)$ discussed in the text.



allows tow numbers n_h^* that are not integers. In practice, the theoretical numbers computed from (T4.10) must be rounded to integers, and the theoretical limit V^* might not be achieved.

We find it helpful to examine first the special case with only one stratum ($m = 1$), which corresponds closely to the analysis in Table 3. The design is then governed by one set of binomial-gamma parameters (p, μ, ν), an area A, and a sample of n tows. It follows from (T4.6)–(T4.7) that

$$(11) \quad \mathrm{E}[\hat{B}] = (1 - p)\mu A,$$

$$(12) \quad \mathrm{V}[\hat{B}] = \frac{1}{n}(\rho^2 + p)(1 - p)\mu^2 A^2,$$

$$(13) \quad \mathrm{CV}[\hat{B}] = \frac{\sqrt{\mathrm{V}[\hat{B}]}}{\mathrm{E}[\hat{B}]} = \sqrt{\frac{\rho^2 + p}{n(1 - p)}},$$

where we express results in terms of the parameter ρ. Increasing the sample size n reduces the variance of $\hat{B}$. Given a target coefficient of variation CV^* in (13), the sample size required to achieve it is

$$(14) \quad n^* = \frac{\rho^2 + p}{1 - p} \frac{1}{(CV^*)^2}.$$

Figure 1*b* portrays the value n^* in relation to (p, ρ) when $CV^* = 1$. It also serves as a general control chart for estimating the sample size needed to attain any target precision CV^* within a single stratum. The sample size indicated in Fig. 1*b* needs only to be adjusted by the factor $(CV^*)^{-2}$. For instance, it must be adjusted upward by a factor of 4 or 100 to meet the targets $CV^* = 0.5$ or $CV^* = 0.1$, respectively.

A simple example illustrates the general idea. Suppose that data from some source, such as a nearby survey or fishery, indicate that about 20% of tows fail to catch the target species and that nonzero tows have a coefficient of variation near 2. We want a biomass estimate $\hat{B}$ with a 20% coefficient of variation

Table 4. Survey parameters, estimates, and optimal design, based on a model with binomial-gamma density parameters (p_h, μ_h, ν_h), area A_h, and cost per tow k_h in each stratum $h = 1, \ldots, m$. Estimates come from independent density measurements $z_{hi} \sim H(p_h, \mu_h, \nu_h)$ with $i = 1, \ldots, n_h$.

Parameters

$$(T4.1) \quad \delta_h = (1 - p_h)\mu_h$$

$$(T4.2) \quad \sigma_h^2 = (1 - p_h)(1 + \nu_h p_h)\frac{\mu_h^2}{\nu_h}$$

$$(T4.3) \quad B = \sum_{h=1}^{m} A_h \delta_h$$

Estimates

$$(T4.4) \quad \hat{\delta}_h = \bar{z}_h = \frac{1}{n_h}\sum_{i=1}^{n_h} z_{hi}$$

$$(T4.5) \quad \hat{B} = \sum_{h=1}^{m} A_h \hat{\delta}_h$$

$$(T4.6) \quad \mathrm{E}[\hat{B}] = B$$

$$(T4.7) \quad \mathrm{V}[\hat{B}] = \sum_{h=1}^{m} \frac{A_h^2 \sigma_h^2}{n_h}$$

Optimal design

$$(T4.8) \quad K = \sum_{h=1}^{m} n_h k_h$$

$$(T4.9) \quad X = \sum_{h=1}^{m} A_h \sigma_h \sqrt{k_h}$$

$$(T4.10) \quad n_h^* = \frac{K A_h \sigma_h}{X\sqrt{k_h}}$$

$$(T4.11) \quad V^* = \min_{n_h} V[\hat{B}] = \frac{X^2}{K}$$

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(CV* = 0.2). In Fig. 1*b*, the point $(p, \rho) = (0.2, 2)$ corresponds roughly to $n^* = 5$, which must be adjusted upward by the factor $0.2^{-2} = 25$ to produce the approximate sample size $n^* = 125$ appropriate for this situation. More precisely, $\gamma^2 = 5.25$ from (T3.4), and $n^* = 131$ from (14). This example highlights the relatively large effort required to achieve a reasonable biomass estimate, given a moderate level of zero tows and highly variable nonzero tows.

Confidence intervals from bootstraps

Our analysis so far has used properties of the binomial-gamma distribution to investigate survey design. Given parameters

$$(15) \quad (p_h, \mu_h, v_h, A_h, n_h), \quad h = 1, \ldots, m,$$

we can also use simulation to produce a data set

$$(16) \quad \{z_{hi}\}, \quad h = 1, \ldots, m, \quad i = 1, \ldots, n_h,$$

suitable for computing a biomass estimate $\hat{B}$ from (T4.4)–(T4.5). The parameters (15) allow us to evaluate a corresponding true biomass B from (T4.3), but the simple estimation formula (T4.5) depends only on the assumption that n_h independent density measurements come from each stratum h with area A_h. The bootstrap offers a means of computing confidence intervals for B that remain independent of explicit parametric assumptions. We can test the statistical validity of these confidence intervals with simulated data from a model in which the true value B is known, such as the stratified binomial-gamma model here.

To be precise, define Q quantile levels by the probabilities $\alpha_j = j/Q$ with $j = 0, \ldots, Q$. For example, if $Q = 20$, then $\alpha_0 = 0, \alpha_1 = 0.05, \alpha_2 = 0.1, \ldots, \alpha_{19} = 0.95$, and $\alpha_{20} = 1$. A confidence quantile B'_j computed from the data set (16) should have the statistical property

$$(17) \quad \text{prob}(B < B'_j) = \alpha_j,$$

where B is a fixed parameter and B'_j is a statistic computed by some algorithm from the data. The statement (17) asserts that, over many data sets, the constant B will lie below the calculated quantile B'_j with probability α_j. If this is true for each level j, then

$$(18) \quad \text{prob}(B'_{j-1} \le B < B'_j) = \alpha_j - \alpha_{j-1} = \frac{1}{Q}.$$

Thus, the interval (B'_{j-1}, B'_j), which varies with each data set, includes B with probability $1/Q$. We define $B_0 = 0$ and $B_Q = \infty$, so that a positive value B necessarily lies in one of the intervals (18).

In order to test the coverage properties (17)–(18) of confidence limits B'_j derived from bootstraps, we start with a fixed set of parameters (15) that define a known true biomass B. These allow us to produce S simulated data sets $\{z_{hi}\}_s$ with $s = 1, \ldots, S$. For each data set s, we generate R bootstrap estimates

$$(19) \quad \hat{B}^*_{rs} = \sum_{h=1}^{m} A_h \hat{\delta}^*_{h,rs}; \quad r = 1, \ldots, R,$$

where $\delta^*_{h,rs}$ denotes the mean density from sampling n_h measurements with replacement from the data for stratum h in simulation s. Quantiles of the bootstrap (19) can be used to define "empirical" limits $B'_{j,s}$, which have the property that a proportion α_j of the bootstrap replicates r lie below $B'_{j,s}$. Appendix A describes two corrections to the empirical limits, associated with bias and acceleration. The essential feature of all these limit calculations is that the results depend only on the data (16) and the stratified biomass estimator (T4.5). Based on S cycles of simulating data, generating bootstraps, and evaluating confidence limits, we calculate the observed coverage

$$(20) \quad \hat{\alpha}_j = \frac{1}{S}\text{count}(B < B'_{j,s}).$$

Comparing $\hat{\alpha}_j$ with α_j allows us to evaluate the success of the bootstrap.

Results

We have already shown from (13)–(14) that $n = 131$ tows produce a 20% coefficient of variation in a single stratum with $(p, \rho) = (0.2, 2)$, the point highlighted in Fig. 1*b*. We explore this case further (Fig. 2) with four smaller sample sizes (n = 50, 20, 10, 5) from simulations with the fixed parameters

$$(21) \quad p = 0.2,\ \mu = 100\,\text{kg} \cdot \text{km}^{-2},\ v = 0.25,\ \rho = 2,\ A = 100\,\text{km}^2,\ B = 8\,\text{t}.$$

Each panel shows 50 independent simulations sorted by ascending median within three groups determined by the 5% and 95% BC_a quantiles. In particular, the group on the left has a 95% quantile smaller than B, and the group on the right has a 5% quantile larger than B.

As the sample size n decreases, more simulations give results in the leftmost group, which includes 22 out of the 50 simulations when $n = 5$ (Fig. 2*d*). If the bootstrap worked properly, this should happen in only 5% (not 22/50 = 44%) of the simulations. For high values of p and ρ, a small survey often produces a low fish catch in every tow, so that the true biomass B lies above the range of observed data. No reasonable statistical method can correct for this problem. On the other hand, large catches can also occur, such as those associated with simulations toward the right in Fig. 2*d*. The range of outcomes in Fig. 2 illustrates the spectrum of possible survey outcomes, even when all parameters (21) remain constant. An apparent significant biomass change between two survey periods might only reflect the limitations of small sample sizes.

To gain better perspective of the example (21), we increase the number of simulations from $S = 50$ (Fig. 2) to $S = 1000$ (Figs. 3–5). With the sample size $n = 200$, we obtain biomass estimates $\hat{B}$ in the range 4–13 t (Fig. 3*a*) that have a coefficient of variation $\text{CV}[\hat{B}] = 0.162$ (Table 5). The distribution of $\hat{B}$ appears fairly normal, perhaps slightly skewed by outliers on the right. Sigmoidal cumulative probabilities (Fig. 3*b*) and linear normalized quantiles (Fig. 3*c*) also suggest that $\hat{B}$ follows a nearly normal distribution when $n = 200$.

For each simulation in Fig. 3, we generate a bootstrap sample with $R = 1000$ replications. Empirical confidence limits

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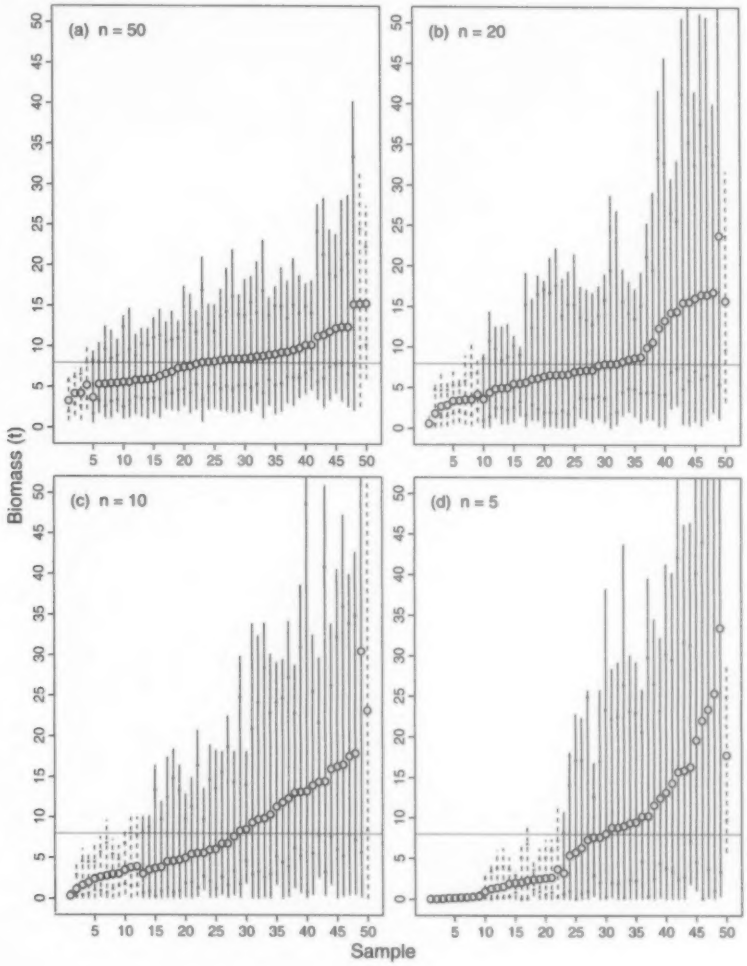
Fig. 2. Estimated biomass from $S = 50$ independent survey simulations, each with $R = 1000$ bootstraps, in a single stratum with parameters (21). A horizontal line represents the true biomass value $B = 8$ t. For each simulation, a vertical line represents the range of bootstrapped estimates $\hat{B}^*$, and a circle designates the median value. Lower and upper tick marks indicate the 5% and 95% BC_a confidence quantiles. Results are sorted by ascending median within three groups: B higher than the 95% quantile (broken lines at left), B between the two quantiles (solid lines), B less than the 5% quantile (broken lines at right). Panels correspond to four sample sizes: (*a*) $n = 50$, (*b*) $n = 20$, (*c*) $n = 10$, (*d*) $n = 5$.



(Fig. 3*d*) taken directly from the bootstraps fail to give the even coverage anticipated in (18). The true value B should lie in each interval with a uniform 5% probability when $Q = 20$, but Fig. 3*d* shows a tendency for B to occur more frequently in the higher level intervals with j near 20. In particular, B lies most frequently in the last interval, where the probability exceeds 8%, that $B'_{19} < B$. Bias correction (Fig. 3*e*) and combined BC_a corrections for bias and acceleration (Fig. 3*f*) give progressively even coverage. Figures 3*g*–3*i* convey a similar message, where points $(\alpha_j, \hat{\alpha}_j)$ lie below the 45° line for empirical confidence intervals (Fig. 3*g*), but move closer to the line with the two corrections for bias and acceleration (Figs. 3*h*–3*i*).

We investigate the example (21) with the sample size reduced to $n = 50$ (Fig. 4), as in Fig. 2*a*. The distribution of $\hat{B}$ is skewed further to the right, the range expands to 3–18 t (Fig. 4*a*), and the coefficient of variation doubles (Table 5). The cumulative

Table 5. True and observed (Obs.) values of the mean, standard deviation, and coefficient of variation from $S = 1000$ estimates $\hat{B}$ produced for Figs. 3–6, based on surveys with N tows.

		$E[\hat{B}]$		$SD[\hat{B}]$		$CV[\hat{B}]$	
Figure	N	True	Obs.	True	Obs.	True	Obs.
3	200	8.00	8.00	1.30	1.29	0.162	0.162
4	50	8.00	7.95	2.59	2.60	0.324	0.327
5	5	8.00	7.89	8.20	8.46	1.025	1.072
6	100	96.60	96.10	16.97	17.34	0.176	0.180

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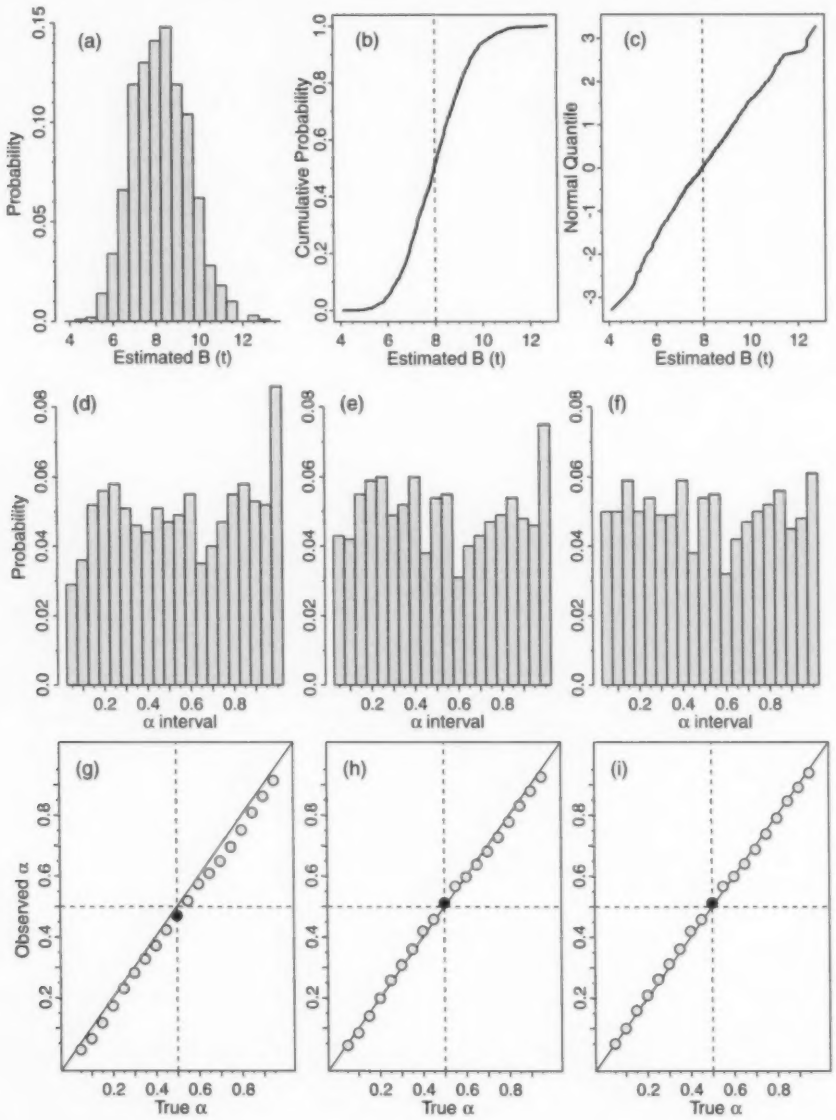
Fig. 3. Results from $S = 1000$ simulations and $R = 1000$ bootstraps for each data set in a single-stratum ($m = 1$) model with parameters (21) and sample size $n = 200$. (**Panels *a–c***) (*a*) Histogram, (*b*) cumulative probability distribution, and (*c*) normal quantiles for the S estimates $\hat{B}$. Vertical dashed lines in Figs. 3*b* and 3*c* denote the true value $B = 8$ t. (**Panels *d–f***) Observed probabilities that the true value B lies in the j^{th} confidence interval (18) based on (*d*) empirical confidence limits, (*e*) limits adjusted only with bias correction, and (*f*) BC_a limits. (**Panels *g–i***) Plot of $\hat{\alpha}_j$ vs. α_j based on (*g*) empirical confidence limits, (*h*) limits adjusted only with bias correction, and (*i*) BC_a limits. Vertical and horizontal dashed lines in *g–i* show median levels at 50%, and solid lines denote the equality $\hat{\alpha} = \alpha$.



probability curve (Fig. 4*b*) becomes asymmetric, and $\hat{B}$ underestimates B with about 58% probability. Normal quantiles (Fig. 4*c*) no longer appear linear. The true value B lies most frequently in the highest confidence interval, although bias correction and acceleration smooth the distribution among lower confidence intervals (Figs. 4*d*–4*f*). Similarly, the two corrections draw points $(\alpha_j, \hat{\alpha}_j)$ closer to the 45° line (Figs. 4*g*–4*i*).

In particular, Fig. 4*i* shows a line of points that fall increasingly below the 45° line as j increases. This pattern relates closely to Fig. 4*f*, where the first 19 confidence intervals each contain the true value B an average of 4.7% of the time, and the final interval has coverage 10.7%. (Thus, $4.7\% \times 19 + 10.7\% = 100\%$.) The slight discrepancies of 0.3% below the theoretical value 5% in the intervals $j = 1, \ldots, 19$ accumulate to pro-

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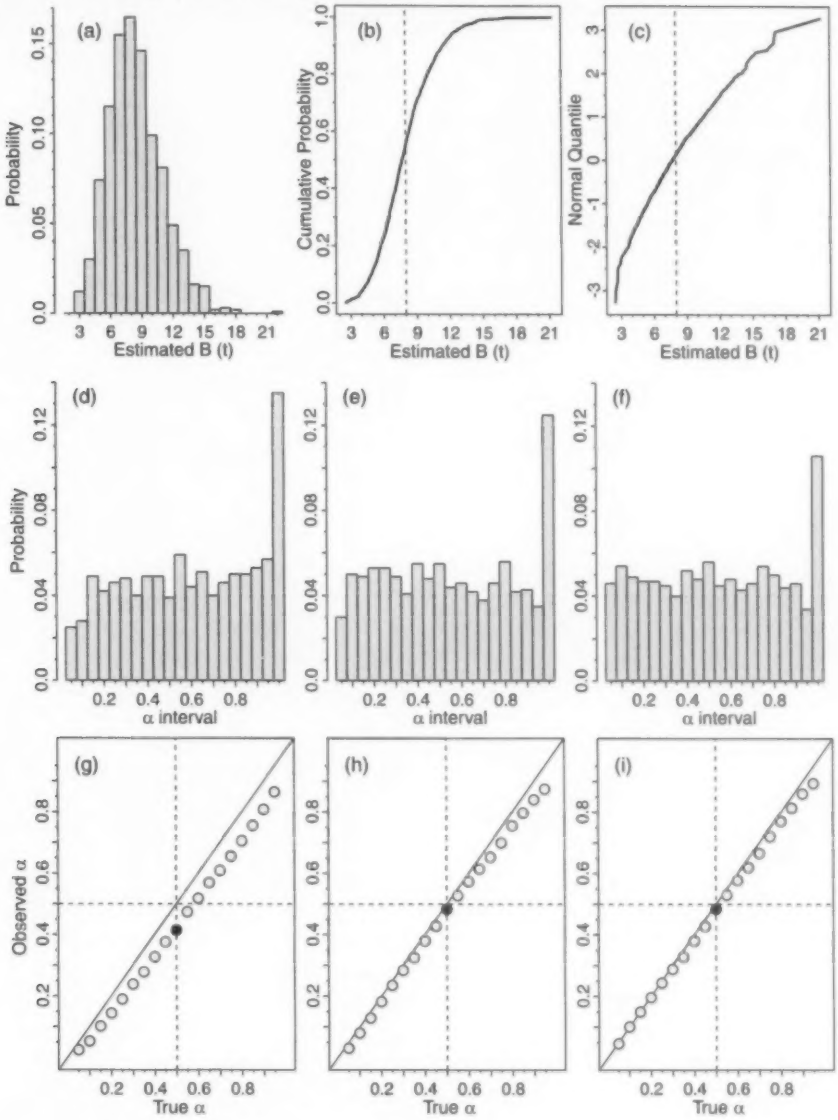
Fig. 4. Results similar to those in Fig. 3, after reducing the sample size to $n = 50$.



duce the linear pattern of points in Fig. 4*i*. The final confidence interval in Fig. 4*f* corresponds to the leftmost group of simulations in Fig. 2*a*, where 4 out of 50 simulations (8%) give a 95% confidence quantile lower than B.

Continuing the investigation of smaller sample sizes, we show the results of a survey with only $n = 5$ tows (Fig. 5), as portrayed earlier in Fig. 2*d*. The problems of skewness and non-normality become pronounced (Figs. 5*a*–5*c*), and $B'_{19} < B$ with probability near 40%, comparable with the proportion $(22/50 = 44\%)$ of simulations in the leftmost group of Fig. 2*d*. Although large estimates $\hat{B}$ are possible, most are low. A data set that gives a low $\hat{B}$ also produces low confidence limits B'_j. The tendency to underestimate B results in points $(\alpha_j, \hat{\alpha}_j)$ well below the 45° line (Figs. 5*g*–5*i*). This reinforces our conclusion from only 50 simulations in Fig. 2. Small samples tend to underestimate biomass, particularly if p is large and ν is small (i.e., ρ is large).

We investigate the extent of this problem across a grid of parameter values (p, ν) for a single stratum survey (Table 6). At each grid point, we compute $\hat{\alpha}_{19}$ from (20) with $Q = 20$ for varying sample sizes n, based on empirical and BC_a definitions of B'_{19}. As suggested by Figs. 2–5, we use the probability that B lies in the highest confidence interval as a measure of bootstrap success, where

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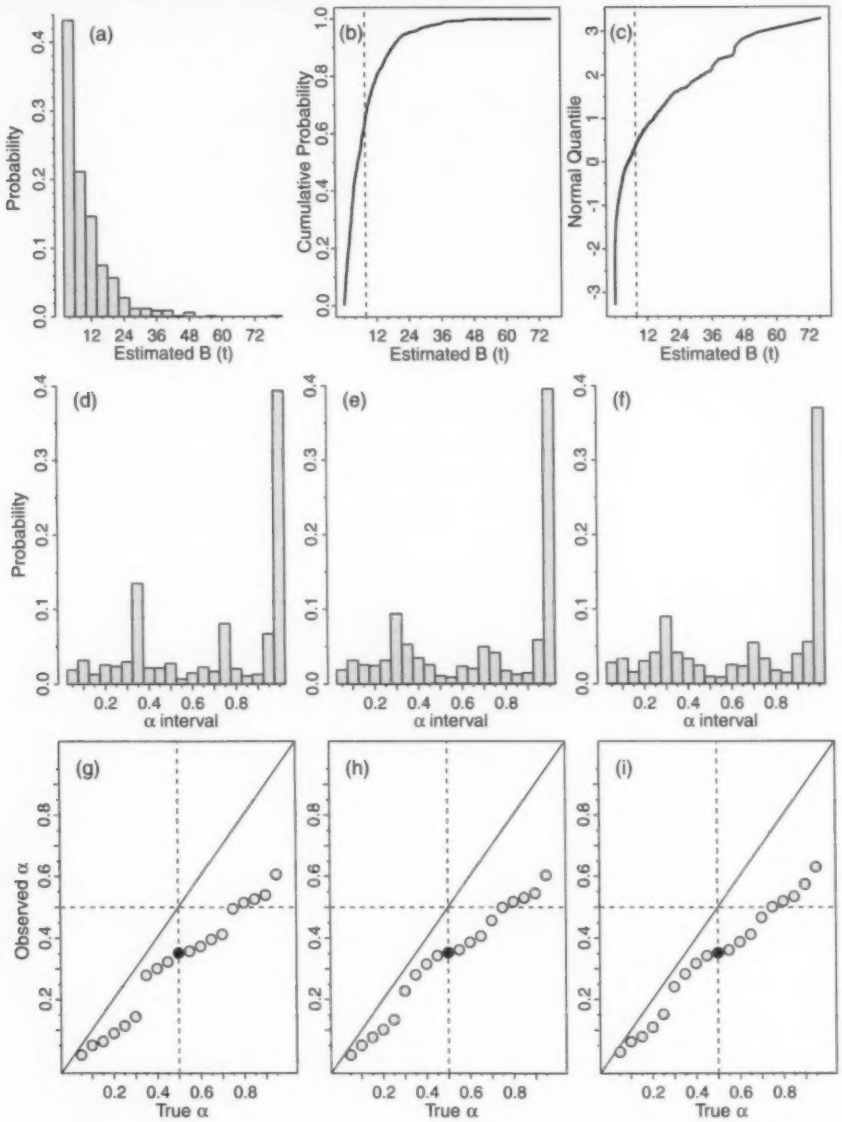
Fig. 5. Results similar to those in Fig. 3, after reducing the sample size to $n = 5$.



$$\beta = 1 - \hat{\alpha}_{19} = \frac{1}{S}\text{count}(B'_{19,s} \le B). \quad (22)$$

Most values $\hat{\alpha}_{19}$ reported in Table 6 lie well below the ideal value 0.95; thus, the observed probability β that B lies in highest interval considerably exceeds the theoretical value 0.05. Generally, BC_a limits perform better than empirical limits, and the results improve with smaller p, larger v, and larger n. These conditions also reduce $CV[\hat{B}]$ in (13).

Our final example portrays a hypothetical survey with six strata (Table 7), where each stratum represents conditions in which groundfish populations might occur. Habitat suitability depends on the target species. In the model, we assume that habitat becomes more suitable as p decreases and μ increases. We choose stratum sizes arbitrarily to give a range from small (100 km^2) to large (1 000 km^2) areas. Table 7 orders the strata by decreasing density δ_h. We reduce v_h as δ_h decreases, following the logic that tows become more variable with declining fish density.

Stratum 1 comprises a small area of prime habitat, with high biomass density μ and a guaranteed catch of at least one target specimen in each tow ($p = 0$). Stratum 2 has a mid-sized area of good habitat where the target species occurs in high densities throughout, but tows sometimes fail to catch the target ($p = 0.1$). This situation might occur if the stratum straddles a depth zone partly outside the optimal range for the target species. Stratum 3 represents a mid-sized area that supports

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Table 6. Based on $Q = 20$ confidence quantiles (17), the observed coverage $\hat{\alpha}_{19}$ corresponding to $\alpha_{19} = 0.95$, as defined by (20). Results for a single stratum ($m = 1$) depend on the parameters (p, v), sample size n, and bootstrap method (Empirical or BC_a). Analysis in this table uses $S = 500$ simulations with $R = 500$ bootstraps for each data set.

		$n = 5$		$n = 10$		$n = 50$		$n = 200$	
p	v	Emp.	BC_a	Emp.	BC_a	Emp.	BC_a	Emp.	BC_a
0.2	0.25	0.63	0.64	0.74	0.79	0.85	0.90	0.91	0.93
0.2	0.50	0.70	0.71	0.81	0.84	0.88	0.92	0.93	0.94
0.2	0.75	0.71	0.73	0.84	0.88	0.91	0.93	0.92	0.94
0.2	1.00	0.78	0.78	0.85	0.87	0.93	0.94	0.94	0.95
0.4	0.25	0.59	0.59	0.69	0.76	0.83	0.90	0.90	0.93
0.4	0.50	0.65	0.63	0.74	0.79	0.88	0.92	0.93	0.94
0.4	0.75	0.72	0.71	0.80	0.85	0.89	0.91	0.92	0.94
0.4	1.00	0.73	0.73	0.80	0.85	0.91	0.94	0.92	0.94
0.6	0.25	0.52	0.50	0.66	0.69	0.79	0.86	0.86	0.91
0.6	0.50	0.62	0.61	0.69	0.74	0.84	0.89	0.92	0.94
0.6	0.75	0.63	0.59	0.74	0.76	0.88	0.93	0.92	0.93
0.6	1.00	0.67	0.63	0.78	0.81	0.93	0.95	0.93	0.95
0.8	0.25	0.30	0.29	0.47	0.46	0.76	0.82	0.84	0.89
0.8	0.50	0.49	0.46	0.61	0.61	0.80	0.87	0.88	0.91
0.8	0.75	0.49	0.47	0.63	0.61	0.86	0.90	0.91	0.94
0.8	1.00	0.56	0.52	0.68	0.65	0.86	0.91	0.94	0.95

Table 7. Biological and design parameters for a survey with $m = 6$ strata and $N = 100$ tows. The cost per tow is assumed the same in all strata ($k_h = 1$). Six rows show the parameters for each stratum h, and a final row shows parameters for the entire survey. The quantities μ, A, and B have units kg·km^{-2}, km^2, and t, respectively.

h	p_h	μ_h	v_h	ρ_h	A_h	δ_h	σ_h	B_h	n_h^*	$SD[\hat{B}_h]$	$CV[\hat{B}_h]$
1	0.0	100	1.0	1.0	100	100.0	100.0	10.0	6	4.08	0.41
2	0.1	80	0.8	1.1	500	72.0	88.2	36.0	26	8.65	0.24
3	0.3	90	0.5	1.4	500	63.0	114.2	31.5	34	9.79	0.31
4	0.5	30	0.3	1.8	1000	15.0	41.5	15.0	24	8.48	0.57
5	0.6	20	0.3	1.8	400	8.0	25.1	3.2	6	4.10	1.28
6	0.9	10	0.2	2.2	900	1.0	7.7	0.9	4	3.46	3.84
					A	Δ		B	N	$SD[\hat{B}]$	$CV[\hat{B}]$
Survey					3400	28.4		96.6	100	16.97	0.18

a patchy target distribution. A high density μ exists within the patches, but elsewhere the target is unavailable ($p = 0.3$). Stratum 4 includes a large area where the target is not abundant but occurs frequently as by-catch. Tows experience low fish densities, at best, and fail to catch the target 50% of the time. Stratum 5 represents a mid-sized area where the target density and availability are lower than that in stratum 4. Finally, stratum 6 has a large area with inappropriate habitat for the target. Consequently, tows seldom catch the target and encounter low densities if they do.

We assume that the cost per tow is the same in all six strata. Effectively, $k_h = 1$, and the survey cost is measured by the number of tows: $K = N$ from (T4.8). Given a budget of $N = 100$ tows, Table 7 shows the optimal sample size n_h^* for each stratum h, calculated from (T4.10). Consistent with the results in Figs. 4–5, the estimate $\hat{B}_h$ has a fairly high coefficient of variation in each stratum. Nevertheless, the combined estimate $\hat{B}$ from the entire survey has lower variability. To use a financial analogy, strata within the survey act like stocks within a portfolio. A diversified combination of volatile stocks has greater stability than each stock alone (Markowitz 1952). Just as a financial manager might adjust share numbers among stocks to minimize portfolio volatility, a groundfish survey allocates tows among strata to minimize variability of the biomass estimate.

Figure 6 shows the outcome of simulations for the survey in Table 7. As in Fig. 4, the estimates $\hat{B}$ appear nearly normal, with a slight skew to the right. Bias correction and acceleration both improve the confidence limits, but even the BC_a limits produce a high value β in (22). A larger sample size $N = 200$ gives better results, similar to those shown in Fig. 3. In general, simulations show the trade-off between sample size and precision in the survey, along with an evaluation of confidence intervals from bootstraps.

Analytical formulas (Table 4) make it easy to compare vari-

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Fig. 6. Results similar to those in Fig. 3, based on the survey design parameters listed in Table 7, with $m = 6$ strata and a total of $N = 100$ tows.

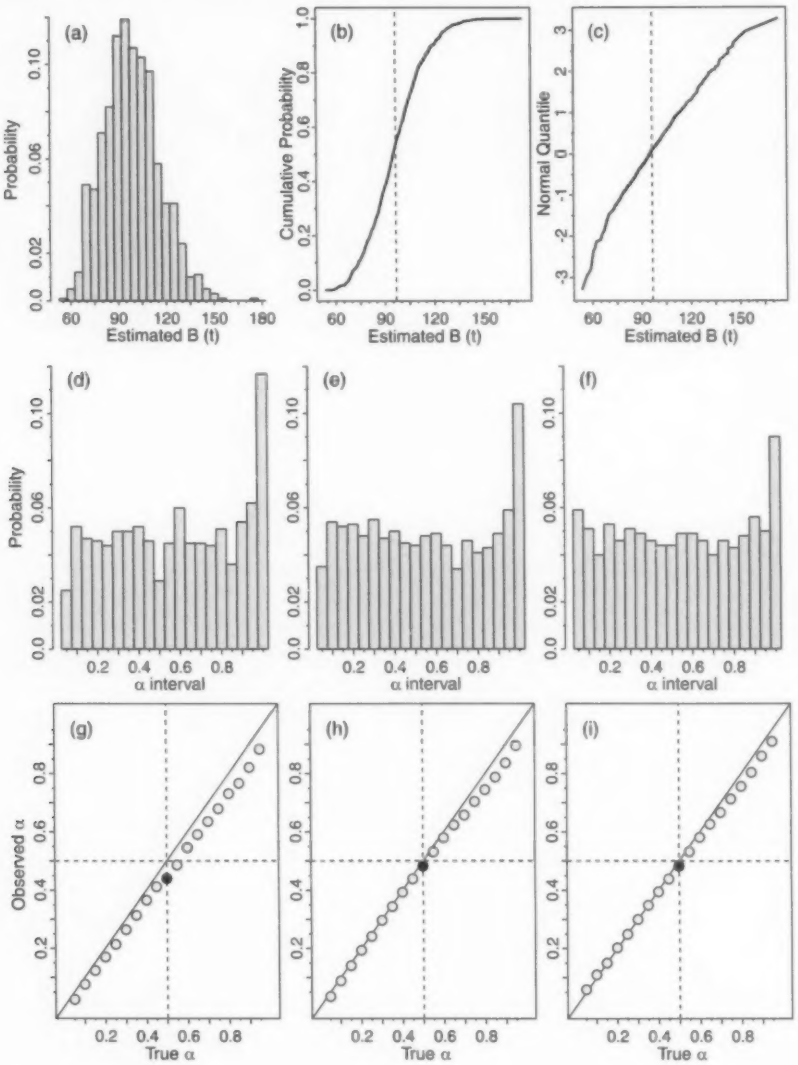

Table 8. Results from five possible survey allocation schemes applied to the population defined in Table 7. Superscripts indicate the method of allocating $N = 100$ tows among six strata: optimal n_h^*, equal number $n_h^=$ in each stratum, n_h^A proportional to area A_h, n_h^B proportional to biomass B_h, n_h^δ proportional to density δ_h. SD and CV denote the standard deviation and coefficient of variation, respectively, achieved for the biomass estimate $\hat{B}_h$ in stratum h with n_h tows. The last line summarizes results for the total biomass estimate $\hat{B}$.

h	n_h^*	SD*	CV*	$n_h^=$	SD=	CV=	n_h^A	SD^A	CV^A	n_h^B	SD^B	CV^B	n_h^δ	SD$^\delta$	CV$^\delta$
1	6	4.08	0.41	16.7	2.45	0.03	2.9	5.83	0.06	10.4	3.11	0.03	38.6	1.61	0.02
2	26	8.65	0.24	16.7	10.80	0.11	14.7	11.50	0.12	37.3	7.22	0.07	27.8	8.36	0.09
3	34	9.79	0.31	16.7	13.99	0.14	14.7	14.89	0.15	32.6	10.00	0.10	24.3	11.58	0.12
4	24	8.48	0.57	16.7	10.17	0.11	29.4	7.66	0.08	15.5	10.54	0.11	5.8	17.26	0.18
5	6	4.10	1.28	16.7	2.46	0.03	11.8	2.93	0.03	3.3	5.51	0.06	3.1	5.71	0.06
6	4	3.46	3.84	16.7	1.69	0.02	26.5	1.34	0.01	0.9	7.16	0.07	0.4	11.13	0.12
	100	16.97	0.18	100	20.75	0.21	100	21.38	0.22	100	18.83	0.19	100	25.71	0.27

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ous tow allocation schemes with the theoretical optimum (Table 8). For the population in Table 7, placing an equal number of tows in each stratum increases SD[$\hat{B}$] from 17.0 to 20.8 t and CV[$\hat{B}$] from 18 to 21%. Similarly, allocating tow numbers n_h in proportion to area A_h, biomass B_h, or density δ_h all increase the coefficient of variation relative to the theoretical optimum. For this example, the least efficient scheme assigns tows proportional to densities (equivalently, catch rates), giving a 27% coefficient of variation in the survey biomass estimate.

Discussion

Our analysis contemplates a stratified groundfish survey, where strata might be defined by spatial regions, depth zones, and other criteria. The calculations (5)–(7) use CPUE data from individual tows to produce an overall abundance index $\hat{B}$. Densities in (5) and (6) act as surrogates for CPUE, weighted relatively by the stratum areas A_h in (7). Consequently, some investigators prefer to interpret the index as a weighted CPUE measurement. Bootstraps generate confidence quantiles by repeatedly applying the estimator (7) to samples drawn with replacement from the observed data. Thus, at the simplest level, a complete analysis of the survey, including a biomass estimate and confidence intervals, depends only on the straightforward logic in (5)–(7). This approach does not require the use of variance formulas from sampling theory.

Following Starfield and Bleloch (1986), we recommend using a model to simulate data collection before embarking on an expensive field program. In this case, tow data typically give a distribution of nonzero density measurements, plus additional zero values. Parameters (p, μ, ρ) capture these characteristics, and the binomial-gamma distribution offers a convenient simulation framework. Bootstrap techniques can be tested with reference to a true biomass B within the simulator. Analytical results aid in tabulating potential outcomes from various tow allocation schemes, using an optimal design as a benchmark for comparison.

In practice, an investigator can use all available data to obtain initial estimates of the parameters (p, μ, ρ) for each stratum, target species, and time period of interest. For example, data might come from previous surveys in similar areas, commercial fishery logbooks, or even informed speculation. In the absence of available data, estimates might come from similar species and strata. The parameters could even be drawn from a parent distribution that reflects variability and correlations. Such a distribution might be estimated from historical data or contrived to explore a range of possibilities.

A spreadsheet or other program can readily implement the formulas in Tables 3 and 4. This makes it possible to explore the results from a chosen set of parameters, as illustrated in Tables 7 and 8. These calculations offer an exploratory framework for investigating the sensitivity of survey statistics, such as CV[$\hat{B}$], to parameter changes and distributions of tow effort. More formally, a Bayes version of the model could progress from a prior distribution of parameter values to the posterior distribution of model outputs.

The model implies a true value B, calculated directly from the input parameters. Thus, a computer intensive process can be used to test coverage properties of bootstrap quantiles, with R bootstraps applied to each of S simulated data sets. Figures 2–6 illustrate such analyses for various model configurations and serve as prototypes for survey planning. In particular, they portray rigorous tests of bias correction and acceleration, with explicit improvements shown for each correction. Our simulations indicate that surveys with small numbers of tows often produce extreme results, either high or low biomass indices. When a survey by chance gives a low biomass estimate, the bootstrap (even after BC_a corrections) gives a 95% confidence quantile that lies below the true value B more than 5% of the time. In other words, data that produce a low biomass estimate often give confidence bounds that don't reveal the true magnitude of the biomass. In a different context, Christman and Pontius (2000) found similar problems with small samples taken from aggregated populations. This doesn't mean, of course, that surveys always produce low estimates. Rather, one should use caution in interpreting the bootstrap quantiles. Our simulation framework helps identify situations in which this problem might occur.

This paper primarily describes survey design for a single target species, where we have compared the reduced variability from stratification with reduced volatility in a diversified financial portfolio. A multiple species survey, with different parameters (p, μ, ρ) for each species, would require a compromise definition of optimality depending on relative species importance. For example, if species x has density variance σ_{hx}^2 in stratum h and total biomass B_x, then the weighted sum of variances

$$W = \sum_x W_x \mathrm{V}[\hat{B}_x] = \sum_{h=1}^{m} \frac{A_h^2 \sum_x (W_x \sigma_{hx}^2)}{n_h} \tag{23}$$

might replace (T4.7) as an objective function to be minimized in selecting the number of tows n_h in stratum h. The result would depend on the relative weight W_x assigned by the investigator to each relevant species x. For example, the choices $W_x = B_x$ or $W_x = 1/B_x$ give highest priority to the species with the most or least biomass, respectively. Mathematically, the function (23) looks almost identical to (T4.7), as can be seen from the equivalent formulation

$$W = \sum_{h=1}^{m} \frac{A_h^2 \tau_h^2}{n_h}, \tag{24}$$

where $\tau_h^2 = \sum_x (W_x \sigma_{hx}^2)$ denotes a weighted sum of species variances in stratum h. Consequently, the optimal design for (23) or (24) is given by (T4.9) and (T4.10) with σ_h replaced by τ_h. In a further extension of this analysis, various species groups might be assessed with multiple surveys funded by a single source. How much should be invested in each survey? Markowitz (1952) deals with a similar problem in a financial context for an investor who holds more than one stock portfolio.

Fish populations exhibit varying degrees of fluidity, depending on the species. We have not directly considered an adaptive design (Thompson and Seber 1996), but we acknowledge that survey design might evolve as new data sets become available. For instance, if a species exhibits an aggregated distribution, estimates of (p, μ, ρ) will differ between areas of aggregation and dispersal. A formal process of revising parameter estimates

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from new data and reapplying the model might lead to revised stratum definitions and better tow allocation schemes.

Previous studies provide considerable evidence that sample allocation schemes have profound effects on design efficiency (e.g., Gavaris and Smith 1987; Smith and Gavaris 1993; Smith and Robert 1998; Ault et al. 1999; Folmer and Pennington 2000; Lunsford et al. 2001). Sinclair et al. (2003) investigate various designs for a multispecies groundfish survey along Canada's Pacific coast. Because an optimal design for one species can be very poor for another, they compromise on a scheme that allocates tow numbers n_h in proportion to area A_h. Comparing this with the theoretical optimum for each species, they calculate the loss of efficiency due to the multispecies compromise. Authors have cited problems from excessive stratification that imposes limits on how tows can be allocated (e.g., Gavaris and Smith 1987). Misallocation can also occur from inappropriate cofactors such as catch rates (Smith and Robert 1998). Lunsford et al. (2001) suggest that aggregated species like Pacific ocean perch (*Sebastes alutus*) are more precisely assessed by adaptive cluster sampling techniques.

In practice, survey estimates are often considered indices, rather than absolute measures of biomass. Following the logic of (1) and (2), we regard the absolute estimate as a useful starting point, but we have cited reasons why it may not be correct. For example, the fishing net may herd some species and scatter others, with a corresponding increase or decrease in the apparent density. Furthermore, different circumstances (such as vessel capability, net design, mesh size, biological cycles, weather fluctuations, and tidal movements) might alter tow selectivity. The model can be extended to include these factors formally, but we do not examine this issue here. We caution, however, against the automatic assumption that ancillary factors act independently. For example, Haigh and Schnute (2003) show significant interactions among depth, year, and latitude in tow data from a longspine thornyhead (*Sebastolobus altivelis*) fishery.

In summary, the binomial-gamma model offers a productive environment for investigating potential groundfish survey costs and benefits. It captures essential characteristics of tows that sometimes fail to capture a target species, but otherwise produce a distribution of density measurements. An application begins by encapsulating available data into three parameters for each species and stratum of interest. Analytical results allow simple "back-of-the-envelope" calculations, which can be expanded into much larger computer explorations. The model adapts easily to new design questions, such as a multispecies survey with the objective function (23). Surveys typically require a team effort with a variety of specialized skills. A simple conceptual model, such as the one described here, offers a framework that can engage all participants in thinking about potential results from a spectrum of possible field activities.

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Appendix A: Bootstrap confidence intervals

The S-Plus 2000 function "bootstrap" has a "group" argument that facilitates subsampling within strata, as in (19). The associated function "limits.emp" gets empirical confidence limits from the bootstrap results. Another function "limits.bca" gives the BC_a confidence bounds for bootstraps from a simple random sample; however, unlike the "bootstrap" function, it does not directly support data stratified into groups.

The BC_a algorithm described by Efron and Tibshirani (1993, pp. 184–186) requires estimating a bias-correction offset $\hat{z}_0$ and an acceleration factor $\hat{a}$. The offset depends only on the bootstrap replicates (19) and the parameter estimate $\hat{B}$ in (T4.5). Let

$$\text{(A.1)}\quad \pi_0 = \frac{1}{R}\text{count}(B_r^* \le \hat{B})$$

denote the proportion of bootstraps that lie below the estimate. Thus, if the bootstrap median matches the estimate, then $\pi_0 = 50\%$; values above or below this level indicate bias. Converting π_0 to a normal variate gives the offset

$$\text{(A.2)}\quad \hat{z}_0 = \Phi^{-1}(\pi_0),$$

where Φ denotes the cumulative normal distribution. The acceleration estimate $\hat{a}$ depends directly on the observed data. In the present context, it requires modification from the formula given by Efron and Tibshirani (1993, p. 186). Specifically, it must be calculated with a stratified jackknife as follows:

$$\text{(A.3)}\quad \hat{a} = \frac{\sum_{h=1}^{m} A_h^3 \sum_{i=1}^{n_h} \left(\hat{\delta}_{h(\cdot)} - \hat{\delta}_{h(i)}\right)^3}{6\left[\sum_{h=1}^{m} A_h^2 \sum_{i=1}^{n_h} \left(\hat{\delta}_{h(\cdot)} - \hat{\delta}_{h(i)}\right)^2\right]^{3/2}},$$

where $\hat{\delta}_{h(i)}$ denotes the jackknife mean with observation i omitted and $\hat{\delta}_{h(\cdot)} = \sum_{i=1}^{n_h} \hat{\delta}_{h(i)}/n_h$. The S-Plus function "limits.bca" calculates $\hat{z}_0$ from (A.1)–(A.2) and allows $\hat{a}$ to be specified externally. We use (A.3) to calculate the "acceleration" argument. Our derivation of (A.3) follows that presented by Smith (1997, p. 630), who obtains a similar result with extra factors for finite population corrections.

Appendix B: Mathematical proofs

Some results in Tables 2–4 require derivation from the assumptions, and we briefly sketch the proofs here. We regard Table 2 as known from the literature. For example, McCullagh and Nelder (1989, p. 287) give the mean and variance of the gamma distribution, along with expressions for the third- and fourth-order cumulants, in terms of the parameters (μ, ν). As mentioned in the text prior to (10), the compound binomial-gamma has mean $\delta = \mathrm{E}[z] = p\cdot 0 + (1-p)\mu = (1-p)\mu$. This proves (T3.2). The corresponding variance can be expressed as

$$\text{(B.1)}\quad \mathrm{V}[z] = \mathrm{E}[(z-\delta)^2] = p(0-\delta)^2 + (1-p)\mathrm{E}_+[(z-\delta)^2],$$

where E_+ denotes the expected value for observations $z > 0$ from the gamma distribution. Furthermore, $(z-\delta)^2 = (z-\mu+\mu-\delta)^2 = (z-\mu)^2 + 2(z-\mu)(\mu-\delta) + (\mu-\delta)^2$; consequently, from (T2.2) and (T2.3),

$$\text{(B.2)}\quad \mathrm{E}_+[(z-\delta)^2] = \mathrm{V}[z>0] + 2\cdot 0\cdot(\mu-\delta) + (\mu-\delta)^2 = \frac{\mu^2}{\nu} + (p\mu)^2.$$

Combining (T2.2) and (B.1)–(B.2) proves the variance formula (T3.3), after some algebra.

To compute optimal sample sizes n_h^* in Table 4, we need to minimize the biomass variance (T4.7) subject to the cost constraint (T4.8). One possible derivation involves a Lagrange multiplier (Thompson 1992, pp. 109–111), but a slightly easier method for this case (Cochran 1977, pp. 97–98) uses the Cauchy-Schwarz inequality

$$\text{(B.3)}\quad \left(\sum_{h=1}^{m} x_h^2\right)\left(\sum_{h=1}^{m} y_h^2\right) \ge \left(\sum_{h=1}^{m} x_h y_h\right)^2,$$

where equality occurs in (B.3) only if

$$\text{(B.4)}\quad x_h = c\, y_h$$

for some constant c independent of h. Consequently, from (T4.7) and (T4.8)

$$\text{(B.5)}\quad \mathrm{V}[\hat{B}]K = \left(\sum_{h=1}^{m} \frac{A_h^2\sigma_h^2}{n_h}\right)\left(\sum_{h=1}^{m} n_h k_h\right) \ge \left(\sum_{h=1}^{m} A_h\sigma_h\sqrt{k_h}\right)^2,$$

where we have applied (B.3) with $x_h = A_h\sigma_h/\sqrt{n_h}$ and $y_h = \sqrt{n_h k_h}$. The condition (B.4) for equality in (B.5) reduces to

$$\text{(B.6)}\quad n_h = \frac{A_h\sigma_h}{c\sqrt{k_h}},$$

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where the constraint (T4.8) determines the constant $c = X/K$ with X calculated from (T4.9). Thus, it follows from (B.5) and (B.6) that $\mathrm{V}[\hat{B}] \geq X^2/K$, where the minimum variance V^* in (T4.11) occurs only if $n_h = n_h^*$ with the optimal n_h^* calculated from (T4.10). This completes the proofs required for Table 4.

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Calculation of untrawlable areas within the boundaries of a bottom trawl survey

Mark Zimmermann

Abstract: A geographic information system (GIS) analysis of 5039 bottom trawl events from U.S. west coast bottom trawl surveys (1977–1998) estimated that the survey area was about 77% trawlable but five of the 30 strata were less than 50% trawlable. Untrawlable areas, by definition, cannot be surveyed with the bottom trawl; however, there has never been a means of identifying and excluding these areas from relative abundance estimates, which are calculated only from hauls completed in the trawlable portions of each stratum. Unknown amounts of untrawlable habitat are a problem for relative abundance estimation in many bottom trawl surveys. This manuscript describes one method of using the bottom trawling events of a survey, such as ripped-up hauls and abandoned stations, to calculate the amount of area that is untrawlable. A comparison of catch rates between undamaged tows and a limited number of damaged tows, which are normally discarded as faulty samples, showed that *Sebastes* catch rates were generally higher in damaged tows. Thus untrawlable areas may have substantial importance on relative abundance estimates of *Sebastes*, the original target species group for this survey.

Résumé : L'analyse au moyen du système d'information géographique (GIS) de 5039 chalutages de fond tirés des inventaires des chalutages de fond de la côte ouest des É.-U. (1977–1998) estime que le chalutage peut se pratiquer sur environ 77 % de la région inventoriée et que, dans cinq des 30 strates, moins de 50 % de la surface est accessible aux chaluts. Les régions inaccessibles aux chaluts ne peuvent pas, par définition, être inventoriées par chalutage; il n'y a cependant jamais eu moyen d'identifier et d'exclure ces surfaces des estimations d'abondance relative qui sont calculées seulement à partir des chalutages réalisés dans les parties accessibles de chaque strate. Les surfaces inconnues d'habitats inaccessibles posent un problème dans les estimations d'abondance dans plusieurs inventaires au chalut de fond. On trouvera ici une méthode qui utilise divers événements au cours d'un inventaire par chalutage, tels que les déchirures de filets et les abandons de stations, pour calculer les surfaces inaccessibles. Une comparaison des taux de capture entre les pêches réussies et un petit nombre de pêches pendant lesquelles les filets ont été endommagés et qui ont été rejetées comme échantillons erronés montre que les taux de capture de *Sebastes* sont généralement plus élevés dans les pêches où il se produit des dommages. L'existence de surfaces inaccessibles peut donc avoir une influence considérable sur les estimations de l'abondance relative de *Sebastes*, le groupe originalement visé par cet inventaire.

[Traduit par la Rédaction]

Introduction

The primary purpose of this project was to demonstrate a method for calculating the amount and location of untrawlable area within a bottom trawl survey by analyzing the history of the National Marine Fisheries Service (NMFS) U.S. west coast triennial bottom trawl surveys (1977–1998). The secondary purpose of this project was to demonstrate that catch rates of *Sebastes* are generally higher in these untrawlable areas, which has confounded attempts to develop precise relative biomass estimates of *Sebastes*. Numerous bottom trawl surveys avoid untrawlable areas or damage their gear in untrawlable habitat, such as in the Gulf of Alaska (Britt and Martin 2001), the Aleutian Islands (Harrison 1993), the eastern Bering Sea (Goddard and Zimmermann 1993), Hawaii (Yoshida 1972), Florida (Pierce and Mahmoudi 2001), and British Columbia (Westrheim et al. 1972), but do not account for the impact of untrawlable areas on relative abundance estimates. Some exploratory trawl surveys have used a combination of navigational chart information, echo-sounding transects, bottom grab samples, and trawl attempts to locate trawlable ground (Hitz et al. 1961; Yoshida 1972). Others have identified untrawlable bottom by underwater video observations (Parker et al. 1983), by summing an assumed area for each reported obstruction (Link 1997), or by reporting positions of obstructions (e.g., Graham 1996). Side-scan sonar and detailed bathymetric mapping produce high-quality habitat maps (Greene et al. 1999; McRea et al. 1999) but cannot always detect the difference between the trawlable and untrawlable locations (Bax et al. 1999). The area that is untrawlable in each bottom trawl survey will depend on the trawl survey methodology and trawl gear used and can best be determined by analyzing the trawl survey history.

The NMFS U.S. west coast triennial bottom trawl survey is a good case study for determining the amount of untrawlable ground because the original target species group was *Sebastes*, which earned their common name of "rockfish" from their tendency to live in rocky areas (first use attributed

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M. Zimmermann.[1] National Marine Fisheries Service, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Bldg. 4, Seattle, WA 98115–6349, U.S.A.

[1]Corresponding author (e-mail: mark.zimmermann@noaa.gov).

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to Dr. W.O. Ayres by Love et al. (2002)), and are known to aggregate in areas that are difficult to trawl (Leaman and Nagtegaal 1987). The original survey plan recommended commercial trawl gear equipped with bobbin roller gear in anticipation of the difficulty of trawling on a rocky substrate (Gunderson 1976). In the first (1977) west coast triennial survey, relative abundance estimates of *Sebastes* were determined to be too imprecise (Gunderson and Sample 1980), and the revised station allocation and stratification designs attempted in the 1980 (Coleman 1986), 1983 (Weinberg et al. 1984), and 1986 surveys (Coleman 1988) did not achieve significant improvements. Subsequent surveys (1989–1998) abandoned *Sebastes* as the primary target species group, and the survey stations were restratified and reallocated for general groundfish species (Shaw et al. 2000). However, the survey has continued to assess the relative abundance of *Sebastes* by trawling in the same areas, and there has never been a formal accounting of the survey area that cannot be trawled. The untrawlable areas may play an important role in relative abundance estimation if these areas are large and if target species have differing catch rates inside and outside of untrawlable areas. The inability of the triennial survey to provide precise relative abundance estimates for *Sebastes* may be related (in part) to that portion of the *Sebastes* populations that inhabit the untrawlable areas. In general, alternative assessment methods show that *Sebastes* spp. in waters off Alaska (O'Connell and Carlile 1993), British Columbia (B.C.) (Richards 1986; Matthews and Richards 1991), Washington (Jagielo et al. 2003), and Oregon (Pearcy et al. 1989) have higher abundances in areas that are difficult to trawl. Thus this work reports a geographic information system (GIS) method for quantifying survey trawlability and compares catch rates from trawlable and untrawlable ground to indicate those areas and those species that are not sampled effectively by the survey trawl.

Materials and methods

U.S. west coast triennial survey

Data were analyzed from eight U.S. west coast triennial bottom trawl surveys conducted from 1977–1998 (Table 1). In general, each survey attempted to trawl at about 600 stratified random stations on the continental shelf and upper slope between southern California and southern B.C. All surveys were fished with nylon (1977–1986) or polyethylene (1986–1998) Nor'eastern bottom trawls equipped with 10-cm rubber disks on the ground-line interspersed with 36- and 46-cm bobbins (1977–1986) or 36-cm bobbins (1989–1998). Surveys used a standard target tow duration of 30 min at 1.54 $m \cdot s^{-1}$ (3.0 knots), covering a distance of ~2.8 km and sweeping about 13–14 m with both trawl types (Coleman 1988). Each catch is standardized by the area swept of the trawl to determine catch-per-unit-effort (CPUE, $kg \cdot ha^{-1}$). The mean CPUE within each stratum is multiplied by the stratum area (ha), including untrawlable areas, to obtain the stratum abundances, which are summed within larger International North Pacific Fisheries Commission (INPFC) statistical areas (Fig. 1). Thus the survey has only analyzed successful bottom hauls to calculate relative abundances, while unsuccessful trawling events are not analyzed. This analysis sought to utilize successful and unsuccessful trawling events, such as hung-up and torn-up hauls and abandoned stations, to identify the amount of trawlable and untrawlable habitat. Therefore, each trawling event was reexamined to determine how it could be used to classify habitat. Each trawling event was then converted into positions, or points, that geographically represented each event, and then these points were used to classify small areas as trawlable and untrawlable.

Performance codes

Portraying the trawl data accurately in a GIS is dependent on the correct classification of the hauls. Originally (1977–1980), there were only four performance codes available for classifying the hauls (Table 2). Good hauls were those with no problems, whereas hauls with hangs, tears, or mechanical (or "other") problems had altered catch compositions and therefore could not be used for relative abundance estimation. Thus these performance codes served a dual purpose: to record what happened during a haul and to determine whether a haul should be used for abundance estimation. In the early years, there was a conflict between these two purposes and a subjective decision had to be made when the tear-up or hang-up was intermediate. There were also several occasions when the tear-up or hang-up was substantial, indicating that the tow should be nullified, and yet the trawl retained a large catch of *Sebastes*, indicating that the tow should be retained. A new performance code was added before the 1983 survey to indicate those hauls with a minor hang-up (Table 2), but this expanded performance coding system was still inadequate. Before the 1998 survey, a more complex system was adopted that served the dual purposes of recording the trawling event (number) and the significance of the trawling event (positive or negative) independently (Table 2).

Standardizing haul performance classification among years

An examination of the handwritten comments on the original data sheets showed numerous contradictions between the reported performance codes and the events that occurred. In general, hauls that were originally coded as unsatisfactory because of mechanical problems (5) were verified and simply given a more descriptive code (–5.1 to –5.9). Those hauls with insufficient catch rates of benthic organisms were reclassified as off-bottom (Zimmermann et al. 2001) if they were originally classified as "good" or "satisfactory". All hauls with any recorded damage (±2.0 to ±2.8), hang-ups (±1.0 to ±1.3), or catch of boulders (±4.1) were reclassified to indicate untrawlable ground. The comments associated with these obstructed hauls were further examined to determine if the obstruction was at the end of the trawl path or at an unknown place along the trawl path, so that the portion of trawl path indicating rough ground was minimized. All hauls that were shortened because of an obstruction or rough ground were separated from the other good hauls for use in charting untrawlable ground, although there is no performance code for classifying them.

Standardizing abandoned stations among years

Stations that were abandoned because of rough or steep ground in each of the surveys were identified for this analysis. There were four substantially different, stratified random

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Table 1. Bottom trawl surveys included in the analysis with the year that each survey was conducted, the general area covered, the bottom trawl gear and material of net construction (n, nylon; p, polyethylene), the vessels that conducted the work, and the agencies that conducted the surveys.

Year	Area	Gear (material)	Vessel	Agency
1977	CA-WA	Nor'eastern (n)	Commando, David Starr Jordan, Tordenskjold, Pacific Raider	NMFS,WDF, and ODFW
1980	CA-BC	Nor'eastern (n)	Mary Lou, Pat San Marie	NMFS
1983	CA-BC	Nor'eastern (n)	Warrior II, Nordfjord	NMFS
1986	CA-WA	Nor'eastern (n & p)	Pat San Marie, Alaska	NMFS
1989	CA-BC	Nor'eastern (p)	Pat San Marie, Golden Fleece	NMFS
1992	CA-BC	Nor'eastern (p)	Green Hope, Alaska	NMFS
1995	CA-BC	Nor'eastern (p)	Alaska, Vesteraalen	NMFS
1998	CA-BC	Nor'eastern (p)	Dominator, Vesteraalen	NMFS

Note: Codes for area covered: BC, British Columbia; WA, Washington; CA, California. Codes for agencies: NMFS, National Marine Fisheries Service; WDF, Washington Department of Fisheries; ODFW, Oregon Department of Fish and Wildlife.

Fig. 1. Idealized strata boundary scheme of the National Marine Fisheries Service U.S. west coast triennial bottom trawl survey. Strata 10–19 are shallow (55–183 m), strata 20–29 are mid-depth (184–366 m), and strata 30–39 are deep (367–500 m). The International North Pacific Fisheries Commission (INPFC) statistical area boundaries are shown as thick, horizontal, black lines on the lefthand side of the map. Trawlability determined from analysis of the 1977–1998 triennials is indicated by shading of strata.

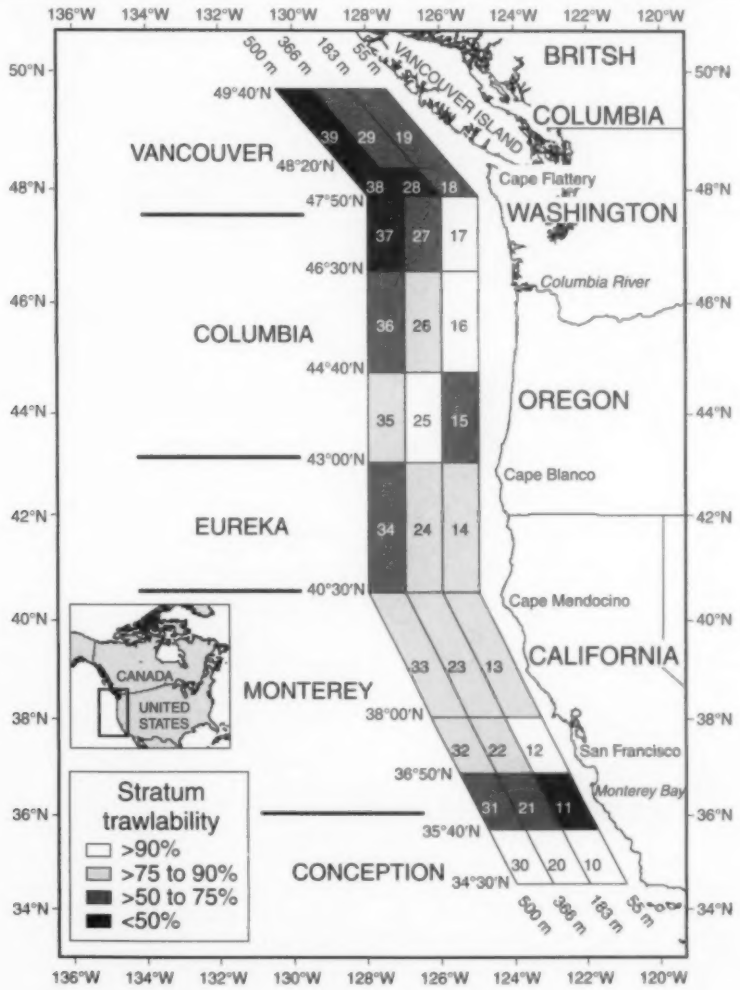

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Table 2. Performance codes used during three time periods of the National Marine Fisheries Service U.S. west coast triennial bottom trawl survey.

Code	Trawling event	Meaning	Use for assessment?
1977–1980			
0	Good performance	No problems	Yes
5	Gear or mechanical problems	Malfunction	No
6	Hung up	Catch affected	No
7	Torn up	Catch affected	No
1983–1995			
0	Good performance	No problems	Yes
1	Hung up	Catch not affected	Yes
5	Gear or mechanical problems	Malfunction	No
6	Hung up	Catch affected	No
7	Torn up	Catch affected	No
1998 and later			
0	Good performance	No problems	Yes
1.1 to 1.2	Hung up	Catch not affected	Yes
2.1 to 2.6	Gear damage	Catch not affected	Yes
3.1 to 3.3	Gear conflict	Catch not affected	Yes
4.1 to 4.6	Caught object	Catch not affected	Yes
5.1 to 5.8	Gear performance problems	Catch not affected	Yes
6.1 to 6.31	Other problems	Catch not affected	Yes
–1.1 to –1.2	Hung up	Catch affected	No
–2.1 to –2.8	Gear damage	Catch affected	No
–3.1 to –3.3	Gear conflict	Catch affected	No
–4.1 to –4.6	Caught object	Catch affected	No
–5.1 to –5.8	Gear performance problems	Catch affected	No
–6.1 to –6.31	Other problems	Catch affected	No

Note: The trawling events that the codes describe, the meaning, and whether or not the trawl was used for assessment purposes are also given.

station patterns used in the survey: 1977, 1980–1983, 1986, and 1989–1998. During each survey, the vessel captain uses the echo-sounder to search for a trawlable path that will come within a 1 nautical mile (M) radius (1852 m) of the station. This search radius was extended to 2.5 M (4630 m) in the 1998 survey. Search time is limited to ~1 h, and numerous stations are abandoned in each survey because the ground is too rough or too steep to attempt a haul. Although the total number of stations abandoned because of untrawlable ground have been reported for the 1989 (Weinberg et al. 1994), 1992 (Zimmermann et al. 1994), 1995 (Wilkins et al. 1998), and 1998 surveys (Shaw et al. 2000), the particular stations abandoned because of untrawlable ground have never been reported for any year. To determine abandoned stations, it was necessary to match up the hauls and stations for each survey in a GIS, while examining the comments recorded for each tow. Stations where the haul was off-station for any reason other than untrawlable ground were not termed abandoned.

Comparing CPUE between trawlable and untrawlable areas

Comparisons were made between CPUEs from untrawlable areas (hung-up, torn-up, boulder, and short hauls) and trawlabie areas (good hauls) to test the null hypothesis that catch rates in trawlable areas were less than or equal to catch rates in untrawlable areas. Ideally, for each species of interest, comparisons would be made between CPUEs within each stratum and year; however, there were few (often zero) shortened and damaged trawls in each stratum–year, exceeding 10 in only one stratum–year. Therefore data from all 8 years were combined for each stratum to increase the sample sizes. Because CPUE data from these trawl surveys are typically right-skewed and contain many zero values, the ranks were tested in a one-tailed Mann–Whitney test.

GIS analysis

Data types

The reclassified triennial survey history was converted into four different types of bottom trawl events for this GIS analysis: abandoned stations; hauls that encountered any obstruction along their paths; hauls that did not encounter any obstructions (good hauls); and hauls with an obstruction at the end of the trawl path. (Hauls with mechanical or "other" problems were not used in this analysis.) Each data type has a different level of confidence associated with it and was given a numerical code in case different events occurred in the same area. Abandoned stations were given the lowest confidence value (1) because the ground was not actually tested with the trawl and because these areas typically contain a mixture of trawlable and untrawlable ground. Next in certainty came those hauls that encountered an obstruction at an unknown place or places along their path (confidence value =

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2). Hauls that did not hang-up or tear-up (good hauls) were given a moderate confidence value (3). Hauls that hung-up or tore-up at or near the end position, or that were shortened because of an upcoming obstruction that was seen on the vessels' echo-sounder, were recorded as two different entities, the trawl path (3) and a point indicating the obstruction at the end of the tow (4).

Converting different data types to point data in a GIS

All GIS data processing was conducted in ArcView (v. 3.2; Environmental Systems Research Institute, Inc., 380 New York St., Redlands, CA 92373-8100) with the goal of converting all trawl data into a sufficient number of points such that a network of 500 m × 500 m cells would be adequately populated. Grid cells of this size were chosen because they correctly represented the navigational accuracy of the trawl data (all positions are recorded at the fishing vessel, not at the net) and because trawling experience indicated that it was reasonable to extrapolate any trawling event (point) to an area 500 m × 500 m. All hauls were converted into lines spanning the distance between the starting and ending positions (Fig 2*a*). These lines were replaced with points ~100 m apart (Fig. 2*b*) so that 500-m grid cells in the trawl path would receive at least one point. Each point was given the appropriate confidence value (e.g., 2, 3, or 4) in order to represent the trawling event from which it was derived.

All abandoned stations (Fig. 2*a*) were converted into circular polygons with radii of 1852 m centered on the station position (Fig. 2*b*), even those in 1998. Each polygon was populated with a regular network of points 500 m apart such that the network of 500 m × 500 m grid cells (Fig. 2c) would be completely populated within the search area of each station. The sum of these populated grid cells is approximately equal to the true area of the circular search area (10.8 km^2). These points were collated into a single file and given the confidence value representing abandoned stations (1).

The point file of the trawling attempts and the point file of the abandoned stations were combined into a single point file and projected into an Albers equal-area conic projection designed for U.S. west coast work (spheroid GRS 80; central meridian –126 longitude, reference latitude 40, first standard parallel 48, second standard parallel 32). All grid cells were classified according to the trawling event with the highest confidence value contained within them (Fig. 2*d*). Grid cells containing no trawling information were deleted.

Triennial survey strata

Since 1995, the survey area has been divided into 30 strata (Fig. 1). The eastern and western boundaries of survey strata are defined by depths, and the northern and southern boundaries are defined by latitudes. Bathymetry lines digitized from paper nautical charts were used as proxies for the depth boundaries in ArcView; however, the true location of the boundary depths varies. Latitudinal borders were created in ArcView, combined with the line files of bathymetry, and converted into polygons representing the survey strata. These polygons of approximated strata, not shown in Fig. 1 because they are too narrow, were used to determine in which strata the trawling events occurred.

Stratum trawlability

The network of grid cells was intersected with the strata boundary file so that whole grid cells or portions of grid cells contained within each stratum boundary were identified (Fig. 2*d*). To determine how much of each stratum was explored for trawlable ground (A_e), the area of those grid cells with point data indicating trawlable (A_t) and untrawlable ground (A_u) was summed within each stratum. The proportion of each stratum that was trawlable (T) was then calculated as $A_t \cdot A_e^{-1}$. The variance of stratum trawlability was estimated by

$$\frac{p \cdot q}{n-1} \cdot \frac{N-n}{N}$$

where p is the proportion trawlable, q is the proportion untrawlable, n is the approximate number of sampling units (500-m squares) explored per stratum, and N is the approximate number of sampling units per stratum. This calculation of variance is most likely an underestimate because it does not take into account the overlap of different data types in the same grid cells.

Impact of grid cell size

Results from this analysis will be dependent, in part, on the size of the grid cells used. The area attributed to abandoned stations will be relatively constant regardless of grid cell size; however, area attributed to haul paths and obstructions will vary by grid cell size. The exact dependence of survey trawlability on grid cell size can not be predicted because of differing amounts of overlap of different trawling events; therefore, the entire analysis was rerun with smaller (100 m and 250 m) and larger (900, 1000, 1500, and 2000 m) grid cells. For reanalyses at grid cells smaller than 500 m, abandoned stations were converted into a network of points 100 m apart so that grid cells as small as 100 m would be fully occupied.

Results

A total of 5039 trawling events were examined for use in describing untrawlable ground. These include 4672 trawling attempts and 367 stations that were examined with echosounders and abandoned because of untrawlable ground. The trawling attempts resulted in 4118 clear trawl paths, of which 265 had obstructions encountered or presumed to exist near the end of the haul path (includes some hang-ups, tear-ups, and all shortened hauls). There were 77 haul paths with an obstruction or obstructions somewhere along their route (the remaining hang-ups and tear-ups) and 467 hauls (other) that were judged unacceptable for mapping untrawlable areas because of gear interference, catch of manmade objects, equipment malfunctions, poor bottom-tending, or other problems. The GIS analysis converted the trawling information into nearly 240 000 individual points that provided information on the trawlability of ~13.5% of the total survey area, with a range of 7.0 to 33.7% per stratum (Table 3). Abandoned stations accounted for 91% of untrawlable ground across the entire survey, whereas torn-up haul paths (6%) and obstructions (3%) were minor components.

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Fig. 2. Conversion of bottom trawling events into trawlable and untrawlable areas. (*a*) Vectors representing clear and damaged trawl paths, obstructions, and abandoned stations are plotted along with stratum boundaries (bathymetry contours). (*b*) Trawl vectors are converted into points approximately 100 m apart along trawl paths. A circular polygon with a radius of 1852 m is drawn around abandoned stations, and the areas within the polygons are populated with a network of points 500 m apart. (*c*) A network of 500 m × 500 m grid cells is created for those locations containing information on trawlability. (*d*) Trawlability information with the greatest degree of certainty populates each grid cell, and grid cells are intersected by strata boundaries. Results for same analysis conducted (*e*) with 250-m grid cells and (*f*) with 1000-m grid cells.

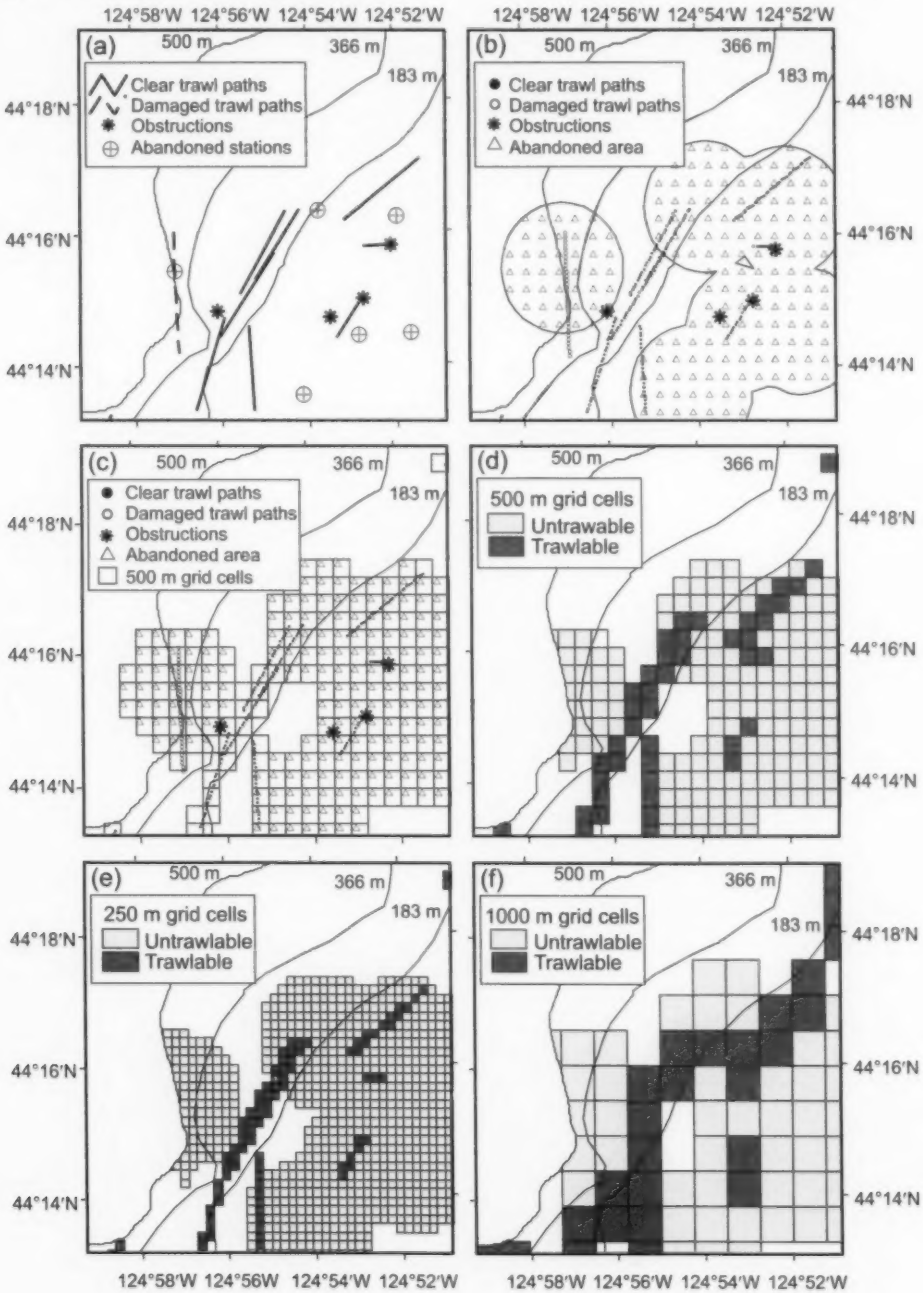

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Table 3. Estimated trawlability of strata in the National Marine Fisheries Service U.S. west coast triennial bottom trawl survey.

Stratum number	Stratum area (km^2)	Areas (km^2) defined by trawling events					Percent stratum sampled	Stratum trawlability	Trawlability variance ($\times 10^{-4}$)
		Good trawl paths	Abandoned stations	Bad trawl paths	Obstructions	Total			
10	1 343.4	126.9	0.0	1.0	2.1	130.0	9.7	0.9765	0.3997
11	718.7	40.2	42.8	0.0	0.1	83.0	11.6	0.4836	6.6701
12	3 735.0	409.3	9.4	0.5	5.0	424.1	11.4	0.9650	0.1767
13	4 724.4	451.4	75.3	0.0	3.8	530.5	11.2	0.8509	0.5309
14	4 090.6	351.6	58.5	1.3	2.3	413.6	10.1	0.8501	0.6930
15	6 250.0	663.1	286.0	9.9	9.4	968.5	15.5	0.6847	0.4710
16	6 014.3	776.9	30.4	1.0	2.3	810.6	13.5	0.9584	0.1063
17	4 410.6	618.8	43.2	11.1	2.4	675.6	15.3	0.9160	0.2411
18	2 282.2	399.5	332.9	21.1	14.9	768.3	33.7	0.5200	0.5389
19	8 587.0	507.3	210.2	19.5	9.8	746.9	8.7	0.6793	0.6661
20	1 089.7	93.7	0.0	0.0	0.0	93.7	8.6	1.0000	0
21	294.0	33.3	26.2	0.0	0.9	60.4	20.5	0.5510	8.1721
22	493.9	69.5	17.4	0.0	0.4	87.3	17.7	0.7958	3.8430
23	1 112.2	180.6	28.6	5.7	0.5	215.4	19.4	0.8381	1.2711
24	1 076.2	147.1	30.4	0.0	1.7	179.3	16.7	0.8206	1.7139
25	1 508.1	226.2	15.3	4.6	1.0	247.2	16.4	0.9152	0.6566
26	2 118.5	252.9	25.2	4.0	1.0	283.1	13.4	0.8933	0.7296
27	536.7	101.6	38.2	2.4	0.7	142.9	26.6	0.7111	2.6420
28	874.6	128.9	124.8	11.6	2.8	268.1	30.7	0.4806	1.6153
29	1 211.2	97.8	48.3	1.3	1.4	148.9	12.3	0.6573	3.3236
30	943.6	84.6	3.2	2.0	0.0	89.8	9.5	0.9420	1.3801
31	302.4	25.5	23.7	0.8	0.2	50.2	16.6	0.5087	10.4317
32	354.4	30.1	7.9	0.0	0.1	38.1	10.8	0.7894	9.7879
33	676.8	83.9	14.4	2.0	0.2	100.6	14.9	0.8342	2.9336
34	736.6	52.4	20.6	0.0	0.2	73.3	9.9	0.7154	6.2793
35	775.0	52.2	5.2	2.9	0.0	60.4	7.8	0.8656	4.4627
36	2 101.2	91.7	53.1	1.3	0.4	146.5	7.0	0.6258	3.7227
37	320.0	15.5	35.4	0.0	0.0	50.9	15.9	0.3041	8.7867
38	241.0	11.3	48.8	0.0	0.0	60.1	24.9	0.1881	4.7888
39	442.2	16.4	23.6	0.0	0.3	40.2	9.1	0.4074	13.7230
Total	59 364.5	6140.3	1679.3	103.9	64.0	7987.5	13.5	0.7687	0.0482

Stratum trawlability

Overall the survey area was estimated to be ~77% (individual strata range of 18.8 to 100.0%) trawlable (Table 3). Five of the 30 strata were less than 50% trawlable, and all of the strata north of the Columbia River were less than 75% trawlable, except for stratum 17 (Fig. 1). In general, shallow strata were the most trawlable, probably because they are the broadest and flattest, and most of the untrawlable areas in strata 15, 18, and 19 were associated with well-known, rocky banks. Stratum 11, the least trawlable of the shallow strata (48%; Table 3), is a combination of very steep and very hard ground where stations are regularly abandoned. Deep strata were the least trawlable because of steepness.

Changes in the usage of performance codes

There have been several changes in the way performance codes are assigned to hauls over the trawl survey history (Table 4). The code indicating mechanical problems (other) was used strictly for its defined purpose in the earliest and latest surveys, but from 1980 through 1986, it was also used to report gear damage. Only four hauls originally coded as suffering hang-ups or tear-ups had comments indicating they had mechanical problems instead. There has been a reduction in the difference between the number of tows classified as damaged and the actual number of tows that were damaged, indicating that survey personnel are more accurately recording what happened during each tow (Fig. 3). The percent of "satisfactory" tows (those with minor problems) has remained relatively stationary since it was introduced into the survey (1983) and has been used variously to record tows with modest damage, shortened tows, and good tows with no recorded problems (may simply lack necessary comments). The category of "satisfactory" was greatly expanded in 1998 (all positive codes), and the increase in the use of this code rate over 1995 is probably linked to the increased utility of the new performance code system. This category was used as a catchall for all sorts of minor trawl events that were worth recording but probably did not affect the trawl performance.

Changes in the occurrence of trawl events

There was an increase in the percent of hauls encountering obstructions from 1977 to 1986 and a decrease since 1986; both the 1995 and 1998 surveys had the lowest per-

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Table 4. Original and revised performance codes assigned to trawls from the National Marine Fisheries Service U.S. west coast triennial bottom trawl survey (1977–1998).

		Original					
Survey	Revised	Good hauls	Satisfactory hauls	Hung-up hauls	Torn-up hauls	Other problem	Not entered
1977 Triennial		665	0	13	1	7	
	Good	560	—	—	—	—	
	Short	2	—	—	—	—	
	Damaged	17	—	12	1	—	
	Other	86	—	1	—	7	
1980 Triennial		571	0	7	14	8	
	Good	381	—	—	—	—	
	Short	1	—	—	—	—	
	Damaged	9	—	7	14	6	
	Other	180	—	—	—	2	
1983 Triennial		537	24	9	16	10	
	Good	494	11	—	—	—	
	Short	3	—	1	—	3	
	Damaged	—	13	8	16	6	
	Other	40	—	—	—	1	
1986 Triennial		515	18	9	20	7	
	Good	482	3	—	—	—	
	Short	9	2	—	—	1	
	Damaged	2	13	9	20	2	
	Other	22	—	—	—	4	
1989 Triennial		514	26	6	5	4	1
	Good	467	5	—	—	—	—
	Short	8	6	—	—	—	—
	Damaged	5	15	6	5	—	1
	Other	34	—	—	—	4	—
1992 Triennial		487	16	11	15	40	
	Good	448	6	—	—	—	
	Short	16	3	—	—	1	
	Damaged	5	6	11	13	4	
	Other	18	1	—	2	35	
1995 Triennial		499	22	5	10	10	
	Good	478	15	—	—	—	
	Short	13	3	—	—	—	
	Damaged	1	4	5	10	—	
	Other	7	—	—	—	10	
1998 Triennial		497	31	2	7	13	
	Good	486	27	—	—	—	
	Short	11	—	—	—	—	
	Damaged	—	4	2	7	—	
	Other	—	—	—	—	13	
Total		4285	137	62	88	99	1
	Good	3796	67	0	0	0	0
	Short	63	14	1	0	5	0
	Damaged	39	55	60	86	18	1
	Other	387	1	1	2	76	0

cent of damaged hauls in the triennial history (Fig. 3). This reduction in the percent of damaged hauls may be linked to increases in the percent of shortened hauls and abandoned stations during the triennial history (Fig. 3), indicating that survey personnel and vessel captains are being more selective about where they try to trawl. The percent at which stations are abandoned declined from 1995 to 1998 and this is due, at least in part, to increasing the search radius around each station from 1.852 to 4.630 km.

Comparing CPUE between trawlable and untrawlable areas

By summing all hauls over the triennial time series, there were only two strata (18 and 19) that had enough damaged

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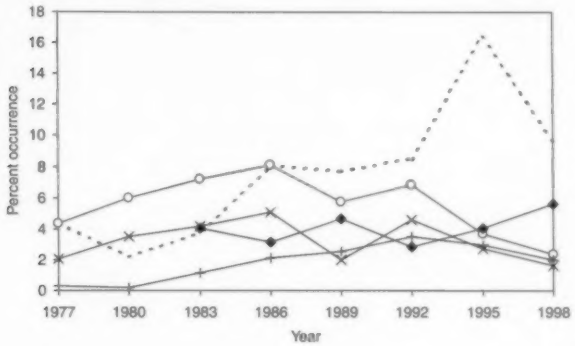
Fig. 3. Percent occurrence of damaged hauls (original (×) and revised (○)), shortened hauls (+), satisfactory hauls (◆), and abandoned stations (broken line) in the National Marine Fisheries Service U.S. west coast triennial bottom trawl survey, 1977–1998. The category of satisfactory hauls, used to indicate those with minor but acceptable problems, was first used during the 1983 triennial survey.



hauls (n = 61 and 50, respectively) that a comparison could be made to the much more numerous undamaged hauls (n = 231 and 300, respectively) within each stratum. Even with summing all hauls over the time series, *Sebastes* species occurred in a minority of all hauls, greatly reducing the number of non-zero samples. The normal approximation (Z) of the Mann–Whitney test statistic was used because group sizes exceeded the group sizes for which the Mann–Whitney test statistic U is calculated (n_1 = 20, n_2 = 40; Zar 1984, table B.10), and Z was compared with $t_{\alpha(1),\infty}$ (Zar 1984). Most Pleuronectiformes had higher catch rates in trawlable areas, whereas most *Sebastes* species had higher catch rates in untrawlable areas. In both strata 18 ($p < 0.05$) and 19 ($p < 0.025$), however, darkblotched rockfish (*S. crameri*) was more abundant in trawlable areas. Results were not definitive for species such as southern rock sole (*Lepidopsetta bilineata*; strata 18, $p < 0.1$; strata 19, $p > 0.5$) and rougheye rockfish (*S. aleutianus*; strata 18, $p < 0.1$; strata 19, $p < 0.05$). Because strata 18 and 19 are adjacent and occur in the same depth zone, trawls from both strata were combined for a more comprehensive test (Table 5). Darkblotched rockfish was more abundant in the trawlable area at the same significance level as Dover sole (*Microstomus pacificus*; $p < 0.005$), a pleuronectiform. Rougheye rockfish was also more abundant in the trawlable area ($p < 0.01$). Two pleuronectiforms, Pacific halibut (*Hippoglossus stenolepis*; $p < 0.1$) and southern rock sole ($p < 0.25$), had marginal significance levels, indicating a lack of preference for either habitat type.

Impact of grid cell size

The estimation of trawlability varied according to the size of grid cells used (Fig. 4). Those analyses with grid cells smaller than 500 m showed a rapid decline in the amount of trawlable ground, with total survey area trawlability decreasing from 76.9% at 500 m to 59.9% at 250 m and to 35.1% at 100 m. Larger grid cells resulted in slightly greater trawlability estimates (84.0% at 1000 m and 88.0% at 2000 m), as decreases in area attributed to abandoned stations were offset by increases in area attributed to obstructions. At the grid cell size of 500 m and smaller (Figs. 2*d* and 2*e*), individual hauls were distinguishable, abandoned stations were relatively round, obstructions were minor components of the landscape, and there was little "bleed through" of trawling events into neighboring strata. Above the grid cell size of 500 m (Fig. 2*f*), individual hauls and abandoned stations were less and less distinguishable, obstructions increased greatly in size, and there was more and more bleed through of trawling events into neighboring strata. The use of 100-m grid cells resulted in hauls and obstructions barely visible in a landscape dominated by abandoned stations (not shown in Fig. 2 because the cells are too small for shading). Therefore, 500-m grid cells were a reasonable geographic representation of the trawling history in a GIS and also minimized the estimate of untrawlable ground.

Discussion

This project demonstrated that there were large untrawlable areas within the U.S. west coast triennial bottom trawl survey strata and that catch rates of several *Sebastes* species were significantly higher in untrawlable habitats within two of the survey strata. The inability of the triennial survey to trawl effectively in rocky areas or to trawl consistently in marginal areas, along with the schooling nature of *Sebastes*, is most likely the reason that the triennial survey was never able to produce precise relative abundance estimates of *Sebastes*. Relative abundance estimates of *Sebastes* from the triennial are not simply imprecise, but inaccurate, because the trawl survey is generally unable to sample the places where *Sebastes* are most abundant. The U.S. west coast triennial survey has always been a habitat-based survey — its limitations have not been quantified before. There is increasing recognition that triennial survey relative abundance estimates for some species, such as yelloweye rockfish, are not useful for assessment purposes (Wallace 2001). Identifying untrawlable grounds may lead to alternative assessment methods in untrawlable areas (Jagielo et al. 2003), which may produce relative abundance estimates with greater precision.

Undoubtedly, there is a problem in utilizing catch rates from damaged hauls for a formal analysis; however, when the damage results in higher catch rates, then the use of these questionable samples seems justified. It is also interesting to note that over one-third of all occurrences of *Sebastes* with the greatest affinity for untrawlable areas (sharpchin, *S. zacentrus*; rosethorn, *S. helvomaculatus*; and yelloweye, *S. rubberimus*) came from the relatively rare damaged hauls. By examining the comments, it is obvious that there has been a bias in the survey to classify torn-up and hung-up hauls as "good" or "satisfactory" if the net retained a large catch of fish; however, this bias should be the same for *Sebastes*, which are assumed to be more abundant in untrawlable areas, and for pleuronectiforms, which are assumed to be more abundant in trawlable areas. There is also a bias in the data because there are no catches from those occasions when the entire net was lost; thus there can be no catch data for the most damaged trawl hauls. There has been a self-fulfilling cultural prejudice in the survey, at times, against recording what was caught in damaged trawl hauls,

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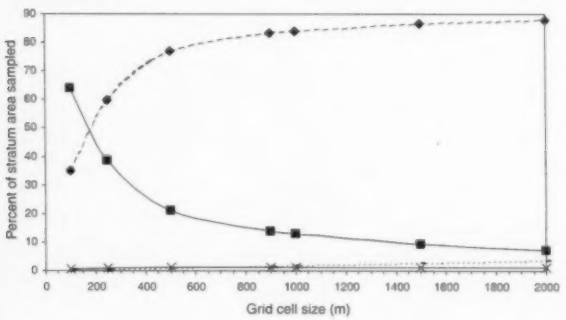
Table 5. Results from the normal approximation of the Mann–Whitney test for determining if the catch-per-unit-effort values in trawlable areas ($CPUE_T$) are less than or equal to catch rates in untrawlable areas ($CPUE_U$) of strata 18 and 19 (H_0: $CPUE_T \leq CPUE_U$; H_a: $CPUE_T > CPUE_U$).

Common name	Scientific name	N_T	N_U	Z value	P
Rex sole	*Glyptocephalus zachirus*	470	63	6.1328	<0.0005
Pacific sanddab	*Citharichthys sordidus*	224	18	5.2244	<0.0005
English sole	*Pleuronectes vetulus*	376	48	4.8691	<0.0005
Flathead sole	*Hippoglossoides elassodon*	231	20	4.5922	<0.0005
Petrale sole	*Eopsetta jordani*	347	42	4.2007	<0.0005
Arrowtooth flounder	*Atheresthes stomias*	470	70	3.5796	<0.0005
Sablefish	*Anoplopoma fimbria*	362	46	3.4300	<0.0005
Darkblotched rockfish	*Sebastes crameri*	123	12	2.7875	<0.005
Dover sole	*Microstomus pacificus*	472	76	2.7252	<0.005
Rougheye rockfish	*Sebastes aleutianus*	65	4	2.5643	<0.01
Pacific halibut	*Hippoglossus stenolepis*	228	34	1.6360	<0.1
Southern rock sole	*Lepidopsetta bilineata*	80	13	0.8470	<0.25
Shortspine thornyhead	*Sebastolobus alascanus*	52	9	0.5191	>0.25
Pacific ocean perch	*Sebastes alutus*	119	25	–0.2760	>0.5
Lingcod	*Ophiodon elongatus*	331	60	–0.4704	>0.5
Redbanded rockfish	*Sebastes babcocki*	46	14	–1.4484	>0.5
Bocaccio	*Sebastes paucispinis*	56	17	–1.6156	>0.5
Yellowtail rockfish	*Sebastes flavidus*	178	44	–2.1947	>0.5
Greenstriped rockfish	*Sebastes elongatus*	190	53	–2.7745	>0.5
Widow rockfish	*Sebastes entomelas*	36	16	–2.7988	>0.5
Canary rockfish	*Sebastes pinniger*	168	57	–4.7720	>0.5
Yelloweye rockfish	*Sebastes ruberrimus*	59	32	–5.4812	>0.5
Rosethorn rockfish	*Sebastes helvomaculatus*	79	48	–7.0848	>0.5
Sharpchin rockfish	*Sebastes zacentrus*	100	54	–7.3133	>0.5

Note: Species names are sorted by the test statistic (Z value), with those more abundant in trawlable areas at the top of the list and those more abundant in untrawlable areas at the bottom of the list. Also shown are the number of hauls with catch of each species from trawlable (N_T, maximum = 543) and untrawlable ground (N_U, maximum = 111) and significance level (P).

Fig. 4. The relationship between the percent of stratum area attributed to different trawling events and the grid cell size used in the geographic information system analysis of overall survey trawlability in the National Marine Fisheries Service U.S. west coast triennial bottom trawl survey, 1977–1998. The percent of stratum area attributed to good trawl paths (◆) is equivalent to the percent of stratum trawlability. Squares (■) represent abandoned stations, dashes represent obstructions, and ×'s represent bad trawl paths.



because it was not deemed appropriate to draw inference from these catches, and this has reduced the number of damaged trawl hauls with catch data even further.

Methodology

The amount of area described as untrawlable in this study is extremely dependent on the bottom trawling methods used for the survey and the GIS methods used to represent those trawling events in the analysis. The amount of untrawlable area would decrease substantially if another bottom trawl survey repeated the station pattern with a trawl equipped with tire gear, if the standard tow distance was reduced, if bottom contact of the trawl was reduced, and if search time and distance was increased. The exact methodology of the west coast triennial survey has changed over time because of changing survey priorities and survey modernization, although our goal has always been to repeat the same trawl survey to the best of our ability. The GIS analysis used the same extent (500 m × 500 m grid cells), which essentially acted as a multiplier, for good haul paths, bad haul paths, and obstructions. Because good hauls were by far the most common event in the survey history, the 500-m grid cells tended to exaggerate the extent of good hauls. Alternatively, a literal interpretation of the area swept by the trawl (13–14 m; Coleman 1988) or the total distance swept between the doors (40 m; Craig Rose, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Bldg. 4, Seattle, WA 98115, U.S.A., personal communication) would have greatly reduced the trawlability estimates. The amount of untrawlable ground attributed to bad hauls was minimized by determining which hauls merely ended at an obstruction. The amount of area at-

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tributed to abandoned stations was also minimized by using a uniform search radius of 1852 m (even for abandoned stations in 1998 when the search radius was 4630 m) and also by only labeling stations as abandoned when there were indications that the station area had been searched (many stations were not trawled for unknown reasons).

Abandoned stations

In the west coast triennial bottom trawl survey, abandoned stations are just points defined by a latitude and longitude, do not occupy a specified area, and there is no official method of accounting for their absence in calculating relative abundance indices. There has never been a consequence of abandoning stations, either in terms of area sampled by the survey or in terms of fish missed. Indeed, some survey strata in 1980 (M. Zimmermann, unpublished data), 1983 (M. Zimmermann, unpublished data), 1986 (Coleman 1988), and 1998 (Shaw et al. 2000) were not successfully sampled, and biomass estimates were simply calculated as zero for those strata. It is not known if abandoned stations are as severe a problem in other bottom trawl surveys, but all bottom trawl surveys that extrapolate results from trawlable grounds to untrawlable grounds are potentially biased. Representing abandoned stations in the GIS analysis as a circle with a radius of 1852 m (area = 10.8 km^2) could be considered an overestimate because it is not possible to search all of the area given the time constraints of the survey and because the search is conducted along the depth contour of the station position. The GIS representation of abandoned stations also could be considered an underestimate because it is only required that any portion of the haul path occur within the search distance and because bottom type likely has a contagious distribution. Search time at each station has not been regularly recorded and is not necessarily a good indicator of trawlability, as stations that are easy to trawl and impossible to trawl both require minimal search time, whereas marginal stations require the most amount of search time, whether or not trawlable ground is eventually found.

Changes in the occurrence of trawl events

The percent of damaged hauls increased from 1977 to 1986, probably because of the changes made in station allocation and stratification, which assigned increasingly more stations to strata in which some *Sebastes* were known to be more abundant. (Over 25% of the stations in the 1986 survey were allocated to stratum 18 (Coleman 1988), which this analysis estimates to be 52% trawlable.) The percent of damaged hauls decreased after 1986 as the survey reallocated stations more evenly throughout the survey area and gradually changed to a more general groundfish species survey. Shortened hauls were rare in the early survey years, but in 1995 and 1998, they were about as common as damaged hauls. Shortened hauls have probably increased because of improvements in standard echo-sounding equipment on the charter vessels, the greater experience of fishing captains in interpreting this technology, and more consistent fishing because of increased gear instrumentation (Zimmermann et al. 2001). It is not known if shortened hauls would provide more accurate or less accurate estimates of fish abundance near untrawlable grounds than hauls that were run into the obstruction and damaged. The percent of abandoned stations was difficult to assess in this project because they have long been a neglected aspect of the survey. There has been an increase in the amount of comments recorded over the survey history, and this probably served to identify more abandoned stations in recent surveys, which might account for some of the increase in abandoned stations in more recent surveys. The increase in recorded comments also indicated that trawling events are being recorded more correctly, as recent surveys have the smallest differences between original and revised estimates of damaged hauls. The increases in abandoned stations and shortened hauls and the decrease in damaged hauls all indicate increasing avoidance of potentially untrawlable ground.

Habitat-related abundance

Comparisons of catch rates between damaged and undamaged hauls were limited to those species with sufficient occurrences in the northern, shallow portion of the survey — other species of interest did not have enough occurrences in strata 18 and 19 to be tested. Both species of rockfish that had higher CPUEs in trawlable areas occur in deeper strata (average depth of occurrence: darkblotched rockfish, 210 m; rougheye rockfish, 270 m; Shaw et al. 2000) than the strata (55–183 m) in which catch rates were analyzed. Therefore, the unexpected affinity of these species for trawlable ground may be due to ontogenetic differences, as the young of both species recruit to soft substrate (Love et al. 1991).

In general, previously published studies agree that most *Sebastes* have higher abundances in areas that are difficult to trawl and that most pleuronectiforms have higher abundances in areas that are easier to trawl. Off northern Vancouver Island, Matthews and Richards (1991) found lower catch rates for Pacific ocean perch (*Sebastes alutus*), greenstriped rockfish (*S. elongatus*), and bocaccio (*S. paucispinus*) using sunken gill nets on trawlable areas, but they also found higher catch rates on untrawlable areas for sharpchin and redbanded rockfish (*S. babcocki*). From submersible observations made off southern B.C., Richards (1986) found that yelloweye rockfish abundance increased with habitat complexity, whereas greenstriped rockfish abundance did not. Jagielo et al. (2003) found higher pleuronectiform abundances in submersible dives conducted on trawlable sites and higher *Sebastes* abundances on untrawlable sites off Washington state. In submersible dives on Heceta Bank off central Oregon, Pearcy et al. (1989) found that several *Sebastes*, including rosethorn, yelloweye, and sharpchin rockfish, were much more abundant in shallow rock and cobble areas than flatfish, whereas flatfish were much more abundant than *Sebastes* in deeper mud areas.

Alternative assessment methods

All methods used for proving the axiom that rockfish are more abundant in untrawlable areas and that flatfish are more abundant in flat areas have their own inherent difficulties. Therefore each alternative assessment method will have to be conducted over both the untrawlable and trawlable ground identified in this study, so that there can be some calibration with the trawl survey. Remotely operated vehicles (ROVs), mini-submersibles, and towed camera sleds have limited visibility ranges for detecting and identifying fish, only exposed fish in a designated viewing area can be counted, area

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swept calculations and underwater navigation are difficult, only small areas can be surveyed, and lights and electronic currents may attract or repel fish. For example, Pearcy et al. (1989) found that canary (*S. pinniger*) and yellowtail (*S. flavidus*) were pelagic, schooling species that were difficult to assess from the submersible and that yellowtail tended to be attracted to the submersible and follow it around. Canary, sharpchin, and yellowtail were the most abundant species in nearby bottom hauls, whereas rosethorn and unidentified schooling and nonschooling rockfish dominated submersible counts (Pearcy et al. 1989). Jagielo et al. (2003) was unable to identify the species of 78% of large (>20 cm) pleuronectiforms and 30% of large rockfish that were counted for analysis, whereas species identification in a trawl survey is much simpler.

Application to other bottom trawl surveys

Other bottom trawl surveys do not account for untrawlable ground, and the methods described in this study may be useful for quantification. For example, in the 1991 NMFS Aleutian Islands bottom trawl survey, between 10 and 20% of all hauls suffered hang-ups or tear-ups, and more than two-thirds of stations in some strata were abandoned because of rough ground (Harrison 1993). First, it is necessary to reinterpret historical data within the context in which it was collected, by using the handwritten comments on the original paper records, so that trawl data from several sources over many years can be standardized. Second, a trawl performance coding system that separately records the trawl event and the meaning of the trawl event is critical for this type of analysis. Third, predefining the consequences of abandoning each station, or accurately recording the search for trawlable ground at each station, would make this type of project much easier. Finally, a GIS is required to quantify the trawlability problem. If possible, CPUEs from damaged and undamaged hauls can be compared.

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The author thanks all of the field biologists, vessel captions, and crews who conducted the numerous surveys mentioned in this project and who dealt with the difficulties of trawling on untrawlable ground, often in rough weather, and always under time constraints. The moral support of numerous colleagues made it possible to undertake this project and the expert GIS advice of Jan Benson and Angie Greig made it possible to bring this work to fruition. The author also thanks Michael Martin for developing the new performance coding system and realizing that trawling events, and the meaning of trawling events, need to be recorded separately. Critical reviews by David Somerton, Michael Martin, M. Elizabeth Conners, Bob Hannah, Chris Rooper, Gary Stauffer, Jean Rogers, and one anonymous reviewer greatly improved the manuscript.

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Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*)

Aaron W. Schrey and Edward J. Heist

Abstract: Microsatellite analysis of the shortfin mako shark (*Isurus oxyrinchus*) revealed very weak evidence of population structure. Samples collected from the North Atlantic, South Atlantic, North Pacific, South Pacific, and Atlantic, and Pacific coasts of South Africa were screened at four microsatellite loci. Multilocus measures of population subdivision were much lower than expected (F_{ST} = 0.0014, P = 0.1292; R_{ST} = 0.0029, P = 0.019). Power analysis revealed that the study had a high level of power (>0.995) to detect the magnitude of F_{ST} expected based on a previous study of mtDNA and assumptions of equal male and female dispersal. Thus, integrating the results from microsatellite- and mitochondrial-based studies may provide evidence for gender-biased dispersal for the shortfin mako.

Résumé : Une analyse des microsatellites chez le mako à nageoires courtes (*Isurus oxyrinchus*) montre peu d'indications d'une structure de population. Quatre locus microsatellites ont été analysés dans des échantillons de l'Atlantique Nord, de l'Atlantique Sud, du Pacifique Nord, du Pacifique Sud et des côtes atlantique et pacifique de l'Afrique du Sud. Les mesures de subdivision de la population basées sur plusieurs locus sont plus faibles que ce à quoi on s'attendait (F_{ST} = 0,0014, P = 0,1292; R_{ST} = 0,0029, P = 0,019). Une analyse de puissance indique que l'étude possède un haut niveau de puissance (puissance >0,995), permettant de détecter la valeur de F_{ST} attendue d'après une étude antérieure de l'ADNmt et les présuppositions de dispersion égale des mâles et des femelles. Ainsi, l'intégration des résultats des études sur les microsatellites et sur les mitochondries pourra peut-être fournir des indices d'une dispersion différente selon le sexe chez le mako à nageoires courtes.

[Traduit par la Rédaction]

Introduction

The shortfin mako (*Isurus oxyrinchus*) is a large pelagic shark that inhabits both coastal and open ocean waters of all tropical and warm temperate oceans (Compagno 1984). Shortfin makos are taken in both commercial and recreational fisheries throughout the world (NMFS 2001). Like many sharks, reproductive characteristics may limit recruitment, causing a susceptibility to overfishing (Holden 1974; Mollet et al. 2000). Therefore, detecting population structure is important for the effective management of this species. The presence of genetic structure is indicative of low levels of migration occurring among stocks and may require certain segments of the shortfin mako distribution to be managed as independent units (Mortiz 1994).

Genetic markers have been used to estimate stock structure in several species of sharks and a larger number of marine teleosts. Generally, there is little, if any, genetic differentiation among samples collected within the continuous ranges of shark populations (summarized in Heist 1999). Recent genetic stock structure studies of sharks include that of Feldheim et al. (2001), who estimated F_{ST} at less than 0.03 for lemon sharks (*Negaprion brevirostris*) between the Northern Hemisphere (Bahamas and Florida) and Atol das Rocas, Brazil, and Pardini et al. (2001), who found no significant differences in microsatellite allele frequencies among white sharks (*Carcharodon carcharias*) between Australia – New Zealand and South Africa but found considerable phylogeographic signal in mtDNA. Conversely, Gardner and Ward (1998) found larger estimates of G_{ST} based on allozyme (mean G_{ST} = 0.113 for seven loci) and mtDNA (G_{ST} = 0.083) data in gummy sharks (*Mustelus antarcticus*) from Australian waters and concluded that stock structure existed for both sexes. To date, most studies of genetic stock structure in sharks have been performed on coastal species; the only pelagic species for which genetic data have been previously published from multiple geographic samples is the shortfin mako. Low levels of genetic stock structure in sharks is consistent with the generally greater amounts of gene flow in marine fishes, which experience few barriers to migration (Ward et al. 1994; Waples 1998). Studies of pelagic teleosts, such as tunas (Ward 1995; Ely et al. 2002), swordfish (Bremer et al. 1996), and billfishes (Buonaccorsi et al. 2001), indicate that where stock structure can be detected using molecular markers, it is generally significant only between, rather than within, ocean basins. The life history traits of the

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A.W. Schrey[1] and E.J. Heist. Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University Carbondale, Carbondale, IL 62901-6511, U.S.A.

[1]Corresponding author (email: aschrey@siu.edu).

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shortfin mako, which include internal fertilization, viviparity with a long (perhaps as long as 18 months) gestation period (Mollet et al. 2000), and production of a small number (four to 16) of precocious offspring ~70 cm long and 2.5 kg at birth (Stevens 1983), are very different from those of most pelagic teleosts, which produce vast numbers of eggs that develop into tiny pelagic larvae. The reproductive mode of sharks like the mako is more similar to that of sea turtles and marine mammals than marine teleosts and makes them extremely vulnerable to overfishing and stock collapse (Musick 1999).

There has been considerable attention over the last few years to the use of molecular markers to identify sex-biased dispersal in vertebrate populations. Techniques used include inferences based on markers with different inheritance patterns (e.g., mtDNA and nuclear DNA) and those based on differences in stock structure or genetic assignment probabilities using biparentally inherited markers (e.g., allozymes and microsatellites) between males and females (Prugnolle and de Meeus 2002). Differences between mtDNA-derived and nuclear DNA derived estimates of stock structure have been used to infer sex-biased dispersal in marine vertebrates, including whales (Palumbi and Baker 1994; Gladden et al. 1999; Lyrholm et al. 1999), sea turtles (Karl and Bowen 1992), and the great white shark (Pardini et al. 2001). In each of these studies, a higher degree of genetic differentiation at mitochondrial vs. nuclear markers was presumed to indicate greater male-mediated vs. female-mediated gene flow. Techniques that use markers with different inheritance patterns have been criticized on the grounds that differences in the mutation rates and effective population sizes among marker types can produce disparate estimates of stock structure among marker types even when gene flow is identical for both sexes (Buonaccorsi et al. 2001; Prugnolle and de Meeus 2002). Estimates based on biparentally inherited markers are potentially more informative if the populations can be sampled in adults following dispersal (Goudet et al. 2002).

In many species of sharks, females migrate to regional or even specific nursery locations for parturition. Although nursery areas are well known for coastal species such as the sandbar (*Carcharhinus plumbeus*; Castro 1993; Merson and Pratt 2001) and blacktip (*Carcharhinus limbatus*; Castro 1993, 1996; Heupel and Hueter 2002) sharks, pelagic species may also pup in time and generalized location that provides adequate food and a refuge from predation, mainly by other sharks. Although little is known about the mating and pupping locations of shortfin makos, Casey and Kohler (1992) observed that young-of-the-year makos occur in the Gulf Stream from the Gulf of Mexico to the Grand Banks and are less common inshore. They hypothesized that makos in the western North Atlantic are born far offshore to protect the young from predation, mostly by other sharks.

There is evidence for population structure in the shortfin mako. Heist et al. (1996) found significant spatial partitioning of mitochondrial DNA (mtDNA) haplotypes using restriction fragment length polymorphism (RFLP) analysis. Pairwise comparisons revealed that a North Atlantic sample was significantly different from samples taken from the South Atlantic, North Pacific, and South Pacific. The researchers concluded that the North Atlantic stock receives a low number of migrants per generation and may require independent management.

Table 1. Geographic locations and numbers of individual shortfin makos (*Isurus oxyrinchus*) collected.

Sample	Location	Number
North Atlantic	East coast, U.S.A.	122
	Azores	23
	Spain	7
		152
South Atlantic	Brazil	20
North Pacific	West coast, U.S.A.	182
	Taiwan	10
		192
South Pacific	New South Wales	43
South Africa	Atlantic and Indian coasts	26
Total		433

To investigate population structure in the shortfin mako at additional loci, we developed multiple microsatellite markers. Microsatellites are short tandem repeats of DNA found multiple times in eukaryotic nuclear genomes. They are highly polymorphic and biparentally inherited codominant markers (Ashley and Dow 1994). The markers developed were used to generate allele frequency data at multiple loci, which were used to estimate levels of population subdivision. Results were compared with the findings of Heist et al. (1996). With large sample sizes and use of multiple nuclear markers, this study may provide insight into population structure of pelagic marine predators, which are inherently difficult to study.

Materials and methods

Shortfin mako tissue samples ($N = 432$) were collected by researchers in the Atlantic, Pacific, and Indian oceans (Table 1). DNA was extracted from heart or muscle tissue following the manufacturer's protocols (DNeasy kit, Catalogue No. 69506; Qiagen Inc., Valencia, Calif.). After extraction, DNA samples were stored at –20°C. This sample set includes most of the samples used by Heist et al. (1996).

Isolation and characterization of loci is described in Schrey and Heist (2002). To determine the genotype of each individual, microsatellite loci were amplified by polymerase chain reaction (PCR). Before the reaction, one primer was radiolabeled with γ^{32}P adenosine triphosphate (ATP) using T_4 polynucleotide kinase. Each 10-μL PCR contained 1–15 ng template DNA, 1× PCR buffer (50 mM KCl, 10 mM Tris HCl pH 9.0, and 0.1% Triton X 100®), 200 μM of each dNTP, 0.14 μM of each primer, 2 mM $MgCl_2$, and 0.1 unit *Taq* DNA polymerase. Thermal profiles had an initial heat denaturation of 95°C for 4 min, followed by 25 cycles of 95°C for 1 min, 56–64°C for 30 s, and 70°C for 30 s (Table 2). Amplified products were separated via electrophoresis on a 6% denaturing polyacrylamide gel 42 cm in length. Each gel was run at ~1600 volts for 3–5 h. Gels were transferred to Whatman 3M blotting paper and dried under vacuum for 1 h. The dried gel was exposed to x-ray film (Kodak X-OMAT) for 1–3 days. Genotypes were determined manually from the x-ray film.

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Table 2. Microsatellite loci used to determine genotypes for the shortfin mako.

Locus	Repeat	Annealing temperature (°C)	Sample size	Number of alleles	Observed heterozygosity	Expected heterozygosity
Iox-01	$(TG)_{19} + (AG)_8$	56	429	46	0.91	0.95
Iox-10	$(GA)_{19}$	64	430	19	0.77	0.82
Iox-12	$(GA)_4 + (GT)_8$	56	421	14	0.82	0.85
Iox-30	$(GT)_6 + (GT)_{14}$	56	424	22	0.89	0.87

Note: Primer sequences and additional details can be found in Schrey and Heist (2002).

Several statistical analysis packages were used for data analysis. GDA version 1d16c (Lewis and Zaykin 2001) was used to calculate observed and expected heterozygosity, to test for conformation to Hardy–Weinberg equilibrium, and to test linkage disequilibrium among pairs of loci within each sample. GENEPOP version 3.3 (an update of version 1.2 described in Raymond and Rousset 1995*a*) was used to calculate allele frequencies and estimates of F_{ST}. GENEPOP calculates a slightly modified version of Weir and Cockerham's (1984) θ to estimate F_{ST}, with significance values calculated in the manner described by Raymond and Rousset (1995*b*). R_{ST} Calc (Goodman 1997) was used to calculate estimates of R_{ST}.

Power analysis was performed following Cohen's (1977) proportion of variance method for *F* statistics. This method determines statistical power at a particular effect size. The effect size is set at the value of the test statistic (here F_{ST}) that is of interest to the study. For this analysis, the effect size was set at the level of heterogeneity detected by Heist et al. (1996). To determine this effect size, data generated by Heist et al. (1996) were re-analyzed with Arlequin version 2.0 (Schneider et al. 2001). Overall and pairwise estimates of F_{ST} were calculated from the RFLP data. The calculated overall F_{ST} estimate was used to determine an effect size of interest for the microsatellite data. This was accomplished by using the resulting overall $F_{ST} = 0.148$ ($P < 0.001$) to estimate the effective migration rate (N_em) under the island model, $F_{ST} = 1(2N_em + 1)^{-1}$. This estimate was transferred to a nuclear-based effect size, also via the island model, where $F_{ST} = 1(4N_em + 1)^{-1}$, resulting in $F_{ST} = 0.0799$. This effect size, which was possible given the loci used in this study, was then used to estimate power. The estimate of power, with df = 4, $\alpha = 0.05$, and $n = 400$ and 450 (bracketing the average $n = 426$), was power > 0.995.

Results

Allele frequencies at each locus were characterized by few alleles occurring at relatively high frequencies and several alleles occurring at lower frequencies (allele frequency data are available from the Data Depository at the National Research Council of Canada).[2] The majority of alleles occurred in multiple populations, although some private alleles were observed. The number of alleles per locus and observed heterozygosities indicate a high level of variation at each microsatellite marker (Table 2). No significant deviations from Hardy–Weinberg equilibrium were observed after sequential Bonferroni adjustment (Rice 1989). Tests for linkage revealed only one statistically significant result, loci *Iox-01* and *Iox-30* ($P = 0.001$, Bonferroni adjusted $\alpha = 0.0025$) in the Coastal South African sample.

Table 3. Overall and individual locus estimates of F_{ST} and R_{ST}.

Locus	R_{ST}	F_{ST}
Iox-01	–0.0009	0.0029
Iox-10	0.0013	–0.0002
Iox-12	0.0189	0.0003
Iox-30	–0.0100	0.0024
Total	0.0029*	0.0014

Note: Asterisk (*) indicates statistical significance at $\alpha = 0.05$.

The estimates of F_{ST} were low and not statistically significant. The multilocus F_{ST} was 0.0014 ($P = 0.1292$) and individual locus F_{ST} results ranged from –0.002 to 0.0029 with no estimate being statistically significant (Table 3). Pairwise F_{ST} estimates, calculated for each pair of populations separately, ranged from –0.0041 to 0.0049 (Table 4). Again, no comparison was statistically significant after sequential Bonferroni adjustment. The multilocus R_{ST} taken over all samples was 0.0029 ($P = 0.019$); individual locus R_{ST} values ranged from –0.01 to 0.0189 (Table 3). Pairwise R_{ST} estimates ranged from –0.0146 to 0.0373 (Table 4). One pairwise R_{ST} estimate, between the North Atlantic and North Pacific, was statistically significant after sequential Bonferroni correction ($R_{ST} = 0.0106$, $P = 0.0034$).

Re-analysis of the RFLP data from Heist et al. (1996) found evidence of genetic heterogeneity that was not detected in the original study of Heist et al. (1996). The overall F_{ST} estimate was 0.148 ($P < 0.001$), with pairwise F_{ST} estimates ranging from 0.025 to 0.347 (Table 5). Four of the pairwise comparisons were statistically significant after sequential Bonferroni adjustment. The previous study had identified significant heterogeneity between the North Atlantic sample and all other samples but did not identify the statistically significant difference between the South Atlantic and North Pacific samples.

Discussion

Although F_{ST} estimates of population subdivision from four microsatellite loci were insufficient to reject a null

[2] Supplementary material may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada. DUD 3506. For more information on obtaining material refer to http://www.nrc.ca/cisti/irm/unpub_e.shtml.

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Table 4. Pairwise estimates of F_{ST} below diagonal and pairwise estimates of R_{ST} above diagonal.

	North Atlantic	South Atlantic	South Africa	North Pacific	South Pacific
North Atlantic	—	0.0202	0.0001	0.0106*	0.0025
South Atlantic	–0.0014	—	0.0097	0.0373	0.0199
South Africa	–0.0041	–0.0023	—	–0.0064	–0.0146
North Pacific	0.0016	–0.0005	<0.0001	—	–0.0051
South Pacific	0.0034	–0.0003	0.0036	0.0049	—

Note: Asterisk (*) indicates statistical significance after sequential Bonferroni correction.

Table 5. Re-analysis of RFLP data reported by Heist et al. (1996) for shortfin mako.

	North Atlantic	South Atlantic	North Pacific	South Pacific
North Atlantic	—	<0.001	<0.001	0.007
South Atlantic	0.347	—	0.113	0.004*
North Pacific	0.173	0.025	—	0.130
South Pacific	0.105	0.112	0.025	—

Note: Pairwise comparisons presented with F_{ST} estimate below diagonal and P value above diagonal. Asterisk (*) denotes statistically significant difference not detected in the previous study.

hypothesis of a single genetic stock for the shortfin mako shark, R_{ST} detected a significant difference in allele length between the North Atlantic and North Pacific. These results indicate that some level of population structure may be present between North Atlantic and North Pacific shortfin mako. However, the heterogeneity detected is inconsistent with the magnitude of heterogeneity observed with mtDNA, and the microsatellite data failed to detect heterogeneity among populations that was previously detected.

The statistical significance of the multilocus R_{ST} estimate and the pairwise comparison between the North Atlantic and North Pacific sample should be viewed with caution. This result appears to be driven by a single significant pairwise comparison between the two samples with the largest sample size, North Atlantic and North Pacific. Although the magnitude of R_{ST} for this comparison is not negligible, it is not supported by the corresponding microsatellite-based F_{ST} estimate and is much smaller than the differences detected between these locations with the mtDNA data. Although it is unlikely that shortfin makos from the North Atlantic and North Pacific form a single panmictic unit, especially in light of the mtDNA data, the small amount of migration necessary to drive the magnitude of F_{ST} and R_{ST} so low that the signal of genetic differences among samples is obscured by the noise inherent in finite sampling require that low F_{ST} and R_{ST} values be viewed with caution (Waples 1998).

The results from both F_{ST} and R_{ST} suggest that gene flow occurs between localities sampled and indicates that shortfin makos move among ocean basins at a rate sufficient to reduce genetic differentiation. These findings conflict with the significant levels of structure found by Heist et al. (1996). The low amount of structure in the microsatellite data raises the possibility that the previous mtDNA study committed a type I statistical error, rejecting a true null hypothesis of panmixia, or the microsatellite data committed a type II error, failing to reject a false null hypothesis of panmixia. Current research has independently found significant levels of structure with mtDNA markers (J. Quattro, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, unpublished data), supporting the findings of Heist et al. (1996) and making it unlikely that a type I error was committed. Power analysis was performed to address the concern that a type II error occurred with F_{ST} estimates from the microsatellite data. The resulting estimate of power (power > 0.995) indicates that there was ample power to detect a similar level of structure as was detected with mtDNA.

Differences in effective population size and mutation rate between markers can lead to inconsistent levels of population structure (Buonaccorsi et al. 2001). The magnitude of the difference between heterogeneity estimates for each marker makes it unlikely that the inconsistency is derived solely from differences in effective population size. Microsatellite-based data may underestimate the amount of genetic differentiation. If the migration rate is low, highly polymorphic microsatellite loci may exhibit downward bias in estimates of genetic differentiation (Hedrick 1999; Balloux et al. 2000). This downward bias, if present in microsatellite data, could cause a contradictory level of population structure to be detected with microsatellites as found by mtDNA. Microsatellite loci generally have a high mutation rate, and the previous mtDNA study indicates a relatively low level of migration. However, the loci used in this study did show a range of variability, and all loci individually and combined failed to detect significant levels of structure.

The different inheritance patterns of the two markers may be used to possibly explain the contradiction. Microsatellites are biparentally inherited, whereas mtDNA is maternally inherited. Population structure detected by mtDNA markers represents limited female dispersal or gene flow (Avise 1995). Microsatellites, alternatively, track both males and females. Therefore, if there is low female dispersal but high male dispersal, it is expected that population structure would be observed with a mtDNA marker but not with microsatellites. This situation could occur if female shortfin mako sharks form structured population segments and males dispersed at a sufficient rate to prevent differentiation at the nuclear markers.

Pardini et al. (2001) reported a similar finding for the white shark, and ongoing studies in our lab indicate similar findings for blacktip sharks between South Carolina and the gulf coast

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of Florida (D. Keeney and E. Heist, Fisheries and Illinois Aquaculture Center, Southern Illinois University, Carbondale, IL 62901-6511, and M. Heupel and R.E. Heuter, Center for Shark Research, Mote Marine Laboratory, Sarasota, FL 34236, unpublished data). Population structure was detected among samples at mtDNA markers, whereas microsatellite analysis failed to detect population structure. Researchers suggested that these findings were evidence for gender-biased dispersal in white sharks, with female white sharks being philopatric and male white sharks possibly making long-distance movements. It appears that a similar situation may be occurring with the mako shark. Unfortunately, we do not possess the DNA samples necessary for estimating sex-biased dispersal using only microsatellite data using techniques such as those described by Goudet et al. (2002). Mature makos and especially mature female makos are an uncommon catch in commercial and recreational fisheries, presumably because of gear selectivity (e.g., bait size) and the tendency for such massive sharks to escape gear designed for smaller fishes (Pratt and Casey 1983). Thus, nearly all of our tissue samples come from immature animals.

Prugnolle and de Meeus (2001) list three hypotheses for differences in male-mediated vs. female-mediated gene flow: resource competition, local mate competition, and inbreeding avoidance. Because shortfin mako populations are large, diffuse, and highly dispersive, we do not believe that any of these mechanisms apply to shortfin makos but instead hypothesize that the tendency of female makos to use generalized oceanic pupping regions reduces female-mediated gene flow among ocean basins. Makos undergo parturition during the spring season in both Northern and Southern Hemispheres (Mollet et al. 2000), thus female-mediated gene flow across the equator would require either the movement of smaller, immature females or for mature females to entrain to an opposite seasonal cycle. If philopatry were an adaptation to ensure that offspring are deposited at a time and place that enhances survival, offspring of females that pupped in the wrong season or location would likely have reduced fitness resulting from reduced survival and perhaps improper imprinting of female offspring that do survive. Male sharks may be more adept at shifting the seasonality of mating behavior to accommodate availability of fertile females, and because mating and parturition are separated by at least 12 to 18 months (Mollet et al. 2000) and perhaps thousands of kilometres, in shortfin mako, males need not be philopatric for pupping areas.

To conclude, these data do not provide strong independent evidence for population structure or the existence of independent management units for the shortfin mako. Thus, the need for independent management of shortfin mako stocks is not supported by the microsatellite data. Caution should be used when using genetic data to adopt a single stock approach to management. Waples (1998) noted that a large number of migrants per generation are required to replenish an overfished stock, and it is difficult to show that a sufficient amount of migration is occurring using genetic data alone.

However, the significant genetic structure detected in the mtDNA data indicate that female shortfin makos may exhibit philopatry for parturition sites, and thus reproductive stocks of makos may exist in the presence of considerable male-mediated gene flow. More detailed studies of the timing and location of parturition including genetic (mtDNA) studies with larger sample sizes and more locations will be needed for a meaningful understanding of population structure in this species.

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Change in biomass of benthic and planktonic algae along a disturbance gradient for 24 Great Lakes coastal wetlands

Sheila A. McNair and Patricia Chow-Fraser

Abstract: We quantified the chlorophyll *a* content of planktonic algae and benthic algae in periphyton on acrylic rods and in epiphyton growing on macrophytes in 24 coastal wetlands in all five Laurentian Great Lakes. Sites were selected to represent a wide range of environmental conditions ranging from nutrient-poor, clear-water marshes with abundant macrophytes to nutrient-enriched, turbid systems devoid of aquatic vegetation. Water quality and species and percent cover of submergent macrophytes were measured in each wetland. Principal components analysis (PCA) showed that total phosphorus, turbidity, and suspended solids, variables associated with human-induced degradation, were most strongly correlated with PC axis 1 (PC1), accounting for 69% of the total variation. The PC1 site score was significantly related to both periphyton and phytoplankton biomass, respectively accounting for 54 and 70% of the total variation in periphyton and phytoplankton data, whereas PC1 only accounted for 18% of the variation in epiphyton biomass. Periphytic and epiphytic biomass were negatively correlated with percent cover and species richness of submergent macrophytes, but phytoplankton biomass was not. We conclude that periphytic and planktonic chlorophyll *a* biomass are good indicators of human-induced water-quality degradation and recommend that both benthic and planktonic algal biomass should be routinely monitored as part of an effective wetland management program.

Résumé : Nous avons mesuré la concentration de chlorophylle *a* des algues planctoniques et benthiques, du périphyton poussant sur des tiges en acrylique et de l'épiphyton croissant sur des macrophytes dans 24 terres humides côtières des cinq Grands Lacs laurentiens. Les sites ont été choisis de façon à représenter une gamme étendue de conditions environnementales, allant de marécages à eau claire avec abondance de macrophytes jusqu'à des systèmes enrichis de nutriments, à eau turbide et sans végétation aquatique. Nous avons déterminé la qualité de l'eau, de même que la composition spécifique et le pourcentage de couverture des macrophytes submergés à chacun des sites. Une analyse en composantes principales (« PCA ») révèle que le phosphore total, la turbidité et les solides en suspension, des variables associées à la dégradation anthropique, sont en forte corrélation surtout avec l'axe PC1 et qu'ils expliquent 69 % de la variation totale. Les positions des sites sur PC1 sont significativement associées à la fois aux biomasses de périphyton et de phytoplancton et elles expliquent respectivement 54 et 70 % de la variation totale des données de périphyton et de phytoplancton; par ailleurs, PC1 n'explique que 18 % de la variation des biomasses de l'épiphyton. Les biomasses du périphyton et de l'épiphyton, mais non celle du phytoplancton, sont en corrélation négative avec le pourcentage de couverture et la richesse en espèces des macrophytes submergés. Nous concluons que les biomasses de la chlorophylle *a* dans le périphyton et le plancton sont de bons indicateurs de la dégradation anthropique de la qualité de l'eau et nous recommandons un suivi régulier de la biomasse des algues benthiques et planctoniques comme élément d'une gestion efficace des terres humides.

[Traduit par la Rédaction]

Introduction

Development of bioindicators to assess the quality of wetland communities has received a great deal of attention in the ecological literature over the past decade (see review by Adamus et al. (2001)). This heightened interest reflects the exceptional value of wetlands as habitat for recreationally important waterfowl, wildlife, and fish and also the great challenge to wetland managers to find appropriate tools to assess and monitor both the quality and quantity of such valuable habitat. A variety of multimetric indicators exist, incorporating the response of particular groups of organisms to environmental conditions (e.g., index of biotic integrity for fish and benthic invertebrates (IBI; Simon 1998; Helgen 2001) and wetland zooplankton index (WZI; Lougheed and Chow-Fraser 2002)).

The literature shows a historic bias towards heterotrophic versus autotrophic bioindicators, and of the autotrophs, vascular plants (emergent, submergent, and floating aquatic macrophytes) have been more often used to assess wetland quality (Adamus et al. 2001) than other primary producers. Only recently has there been interest in using algae as wetland bioindicators (McCormick and Stevenson 1998; Stevenson et al. 2001), even though they are commonly used as bioindicators

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S.A. McNair[1] and P. Chow-Fraser. McMaster University, Department of Biology, 1280 Main St. West, Hamilton, ON L8S 4K1, Canada.

[1]Corresponding author (e-mail: mcnairs@mcmaster.ca).

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of environmental quality in streams and rivers (Lowe and Pan 1996). This development is appropriate given that total algal production in wetlands varies widely and may be equal to or greater than that of vascular plant production under some conditions (Goldsborough and Robinson 1996; Wetzel 1996). Their high productivity, small size, and rapid turnover make algae an important food source at the bottom of many complex wetland food webs (Keough et al. 1996; Lamberti 1996).

Algae may be benthic (including periphyton that grow attached to any submerged surfaces and epiphyton that grow attached to aquatic plants) or planktonic (phytoplankton that are suspended in the water column) (Goldsborough and Robinson 1996). Unlike vascular plants, which obtain most of their nutrients from the sediment, algae respond directly to changes in nutrients in the water column, utilizing them directly (phytoplankton) or by scavenging nutrients in the benthic algal biofilm (Wetzel 1996). This makes them ideal organisms for monitoring nutrient enrichment resulting from altered land uses. Limnologists have capitalized on this and have developed useful indicators of lake trophic status by developing indices that measure planktonic chlorophyll *a* content in both algae-dominated (Dillon and Rigler 1974; Carlson 1977) and macrophyte-dominated systems (Canfield et al. 1983). In lotic environments, however, periphytic algae, often the dominant primary producers (Lamberti 1996), are the organism of choice because they are relatively immobile and respond quickly to environmental stress (Lowe and Pan 1996; U.S. Environmental Protection Agency (EPA) 2000). Similarly, a periphyton index of biotic integrity and a biological index of phosphorus availability have been proposed for the Florida Everglades because wetlands are hydrologically very dynamic and are subject to sudden influxes of sediment and nutrients (McCormick and Stevenson 1998).

To date, few algal studies have been carried out in coastal wetlands of the Laurentian Great Lakes. These ecosystems are important to study because of the tremendous biodiversity values associated with them (Chow-Fraser and Albert 1999) and because significant wetland destruction has already occurred along most of the Great Lakes shoreline, especially those associated with the lower lakes (Whillans 1982). Many of the remaining wetlands are degraded, having lost a substantial portion of their submergent plant community (Klarer and Millie 1992; Chow-Fraser et al. 1998; Lougheed et al. 2001). In such wetlands, planktonic algae make up a relatively large proportion of the net primary production (Goldsborough and Robinson 1996; Chow-Fraser 1999). By comparison, in undisturbed wetlands in which the submergent vascular plant community is diverse and robust, the planktonic algal component is relatively small and epiphytic algae are dominant (Goldsborough and Robinson 1996).

From Chow-Fraser's (1998) conceptual models of "healthy" and "degraded" wetland ecosystems, we offer the following generalizations. In healthy systems, there is abundant aquatic vegetation, which reduces turbidity by trapping sediment and provides substrata for epiphyton and habitat for benthic invertebrates. Nutrients entering the watershed are taken up by plants and algae, but planktonic and benthic algal biomass are kept low by zooplankton and benthic grazers. Grazer populations in these systems support a diverse fish community of planktivores and benthivores that are controlled by piscivorous fish. In contrast, degraded systems have little aquatic vegetation and are turbid and nutrient-rich, producing high planktonic and benthic algal biomass that further reduces light for germination of aquatic plants. Without aquatic vegetation, the habitat is suitable only for benthivores and planktivores, and as a result, grazers are kept in low numbers, and phytoplankton and periphyton proliferate. Epiphyton biomass is low because there are few vascular plants to serve as substrate. Based on these two models, we predict that benthic algal biomass (measured as chlorophyll *a* content in periphyton on artificial substrata and epiphyton on submergent vascular plants) and planktonic algal biomass (measured as chlorophyll *a*) will increase as wetlands become degraded (as measured by deterioration in water quality). Secondly, we predict that biomass of both epiphyton and periphyton will be inversely related to areal cover of submersed aquatic vegetation because Lougheed et al. (2001) found that submergent plant species richness and density were low in oligotrophic wetlands, increased as nutrients increased, but declined when light became limiting as the result of increased sediment loading.

To test these predictions, we sampled 24 wetlands spanning a range of environmental conditions from extremely clear, nutrient-poor systems with abundant submersed aquatic vegetation to turbid, nutrient-enriched systems devoid of submergent plants, and we compare how the chlorophyll *a* biomass of periphyton (CHL_{peri}), epiphyton (CHL_{epi}), and phytoplankton (CHL_{phyto}) change across this disturbance gradient. By selecting wetlands that occur in all five Great Lakes, we hope that results from this study will be widely applicable and become an important contribution to the development of basin-wide bioassessment tools for Great Lakes coastal wetlands.

Methods and materials

Study sites

Twenty-four coastal wetland complexes throughout the Great Lakes were sampled (Fig. 1). Sites were deliberately chosen to represent a gradient from degraded to pristine based on water-quality information collected in previous years (Lougheed et al. 2001; P. Chow-Fraser, unpublished data). Wetlands from all five Great Lakes are represented, 13 in Canada and 11 in the U.S. There are 11 sites from the upper lakes (Superior, 4; Michigan, 3; Huron, 4) and 13 from the lower lakes (Ontario, 8; Erie, 5) representing a variety of hydrogeomorphic wetland types (Table 1; Chow-Fraser and Albert 1999). Differences in environmental conditions and site types were purposely included so that any significant trends that emerge from this study could be widely applicable throughout the Great Lakes shoreline, without qualification to site type.

Field methods

All field sampling was completed between 24 May and 16 August in 2000 and 2001 between 1000 and 1600 hours. The time period for sampling each wetland is shown in Table 1.

Benthic algae: periphyton

We followed the methods outlined in Goldsborough et al. (1986) to sample periphyton with artificial substrata (clear

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Fig. 1. Map of the Laurentian Great Lakes of North America showing the location of the 24 wetlands sampled for this study in 2000 and 2001. Two-letter codes identify each wetland location and are listed in Table 1. Two wetlands are located at Long Point in Lake Erie (LP), Long Point Inner Bay (LPIB), and Long Point Big Rice Bay (LPBR), but only LP is shown on the map.

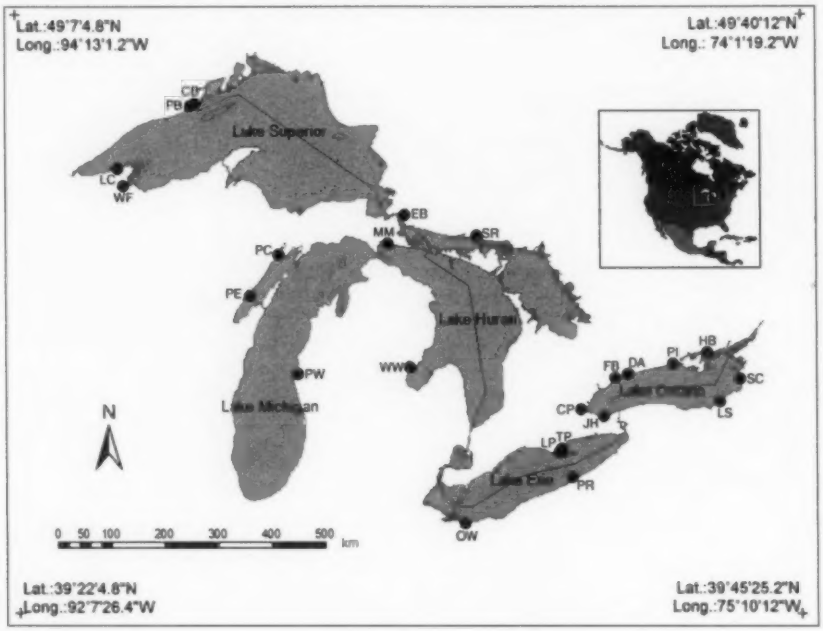

Table 1. Summary of 24 wetlands and sampling period included in this study.

Lake	Wetland, state or province	Wetland code	Wetland type	Sampling period	
				Start	Finish
Superior	Cloud Bay, Ont.	CB	Lacustrine	07/17/01	08/13/01
Superior	Pine Bay, Ont.	PB	Lacustrine	07/19/01	08/14/01
Superior	Lost Creek, Wis.	LC	Estuarine	07/17/01	08/16/01
Superior	West Fish Creek, Wis.	WF	Estuarine	07/17/01	08/16/01
Michigan	Portage Creek, Wis.	PC	Lacustrine	06/15/01	07/10/01
Michigan	Peshtigo Marsh, Wis.	PE	Estuarine	06/14/01	07/10/01
Michigan	Pentwater, Mich.	PW	Estuarine	06/16/01	07/11/01
Huron	Echo Bay, Ont.	EB	Riverine	07/16/00	08/15/00
Huron	Spanish River, Ont.	SR	Riverine	07/15/00	08/15/00
Huron	Mismer Marsh, Mich.	MM	Lacustrine	07/16/00	08/16/00
Huron	Wigwam Bay (Pine River), Mich.	WW	Lacustrine	06/17/01	07/12/01
Ontario	Hay Bay, Ont.	HB	Lacustrine	06/19/00	07/13/00
Ontario	Presqu'Ile, Ont.	PI	Lacustrine	05/29/01	06/27/01
Ontario	Darlington, Ont.	DA	Lacustrine	05/30/01	06/28/01
Ontario	Frenchman's Bay, Ont.	FB	Lacustrine	05/30/01	06/28/01
Ontario	Sandy Creek, N.Y.	SC	Estuarine	06/21/01	07/18/01
Ontario	Little Sodus, N.Y.	LS	Estuarine	06/19/01	07/18/01
Ontario	Cootes Paradise, Ont.	CP	Estuarine	06/27/00	07/24/00
Ontario	Jordan Harbour, Ont.	JH	Estuarine	06/30/00	07/31/00
Erie	Turkey Point, Ont.	TP	Lacustrine	06/07/01	07/05/01
Erie	Long Pt., Big Rice Bay, Ont.	LPBR	Lacustrine	06/06/01	07/04/01
Erie	Long Pt., Inner Bay, Ont.	LPIB	Lacustrine	06/06/01	07/05/01
Erie	Presque Isle, Pa.	PR	Lacustrine	05/23/01	06/20/01
Erie	Old Woman Creek, Ohio	OW	Estuarine	05/24/01	06/21/01

Note: Wetland types are according to Chow-Fraser and Albert (1999). Sampling period dates provided in month/day/year format.

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acrylic rods, 0.6 cm diameter, 90 cm long). Each rod was prescored at 5-cm intervals (to allow subsamples from various depths to be easily taken) and then cleaned with alcohol to remove oils deposited through handling. At each wetland, rods were inserted vertically into the sediment in either four blocks of five rods each or five blocks of four rods each (rods approximately 1 m apart, arranged in a line). Blocks were distributed throughout the wetland area within submergent macrophyte beds if submergent plants were present. Rods were placed in areas of water depth from 40 to 70 cm and at least 3 m from emergent vegetation to prevent shading effects. Samples were collected after ~4 weeks of colonization time. Earlier experiments showed that periphyton chlorophyll *a* biomass reached a peak between 3 and 5 weeks at the test sites and then began to senesce (S. McNair, unpublished data). One 5-cm sample was collected from each rod at a depth of 10–15 cm using cutting pliers. Each sample was wrapped in foil, stored on ice in the field, and then frozen until analyzed for chlorophyll *a* content back in the lab.

Benthic algae: epiphyton

Epiphyton on submergent macrophytes were sampled at the end of the periphyton field incubation period, described above at all sites, and 17 of the 24 sites were also sampled at the beginning of the incubation period. One sample of leaf and (or) stem tissue was collected at each periphyton block from a depth of 10–20 cm and stored in the dark at 5°C before returning to the lab for analysis. If no submergent plants were present, stems of floating or emergent species were collected for epiphyton analysis.

Planktonic algae: phytoplankton

Phytoplankton samples were collected at each wetland complex at the end of the periphyton incubation period using a 1-L Van Dorn bottle in an area devoid of submergent plants. Water samples collected at mid-depth were stored in brown polyethylene bottles and kept at 5°C before processing.

Submergent vascular plants

The submergent macrophyte community was surveyed within each periphyton block using a 0.75 m × 0.75 m floating PVC (polyvinyl chloride) quadrat. Percentage cover of submergents within the quadrat was estimated one time by direct observation at the surface and by raking the bottom if the vegetation was dense or if water turbidity was high. All plant species present in the quadrat and within the periphyton blocks (approximately 1 m × 5 m) were noted. Plants were identified to species where possible and always to genus using Voss (1972) and Newmaster et al. (1997). Species that could not be identified in the field were collected and dried in a plant press for later identification. Several *Potamogeton* species with slender leaves that were not identified to species because of absence of flowering or fruiting structures were grouped into *Potamogeton* spp.

Physico-chemical conditions in wetland

Water samples for nutrient and suspended solids analysis were collected at the end of the incubation period using a 1-L Van Dorn bottle at mid-depth in a vegetation-free area. Samples were stored in acid-washed Nalgene® bottles and kept in the dark at 5°C before returning to the lab for analysis. Physical measurements of ambient water were made at the beginning and end of the field incubation period at each wetland complex. Measurements made at the same time as the water collection for nutrient analysis were used in statistical analyses. Turbidity was measured with a Hach 2100P turbidimeter (Hydrolab-Hach, Loveland, Colo.). Temperature, pH, dissolved oxygen (DO), conductivity, and total dissolved solids (TDS) were measured with Hydrolab multiparameter probes (Hydrolab-Hach; H20 sonde or Mini sonde 4a with a Surveyor; or Quanta). Light measurements were made with a LI-COR photometer (Lincor, Lincoln, Nebr.) equipped with spherical submersible sensor at 19 sites in a vegetation-free area. Rain prevented accurate measurement at the other five wetlands. Sediment samples were collected using an Ekman grab sampler for determination of organic material, total phosphorus, and exchangeable ammonia content.

Laboratory methods

CHL_{peri}, CHL_{epi}, and CHL_{phyto} were determined by pigment extraction in 90% acetone and spectrophotometry (American Public Health Association 1992). Periphyton rod sections were placed in 90% acetone in the freezer for 24–96 h. Samples were centrifuged, and chlorophyll *a* content was determined by measuring absorbance with a Milton Roy 301 spectrophotometer (Fisher Scientific, Toronto, Ont.) before and after acidification (to account for phaeophytin pigments). Epiphyton was removed from vascular plant samples by shaking in 250 mL of distilled water in a 500-mL Nalgene® bottle for 5 min at a rate of 80 beats/min (method after V. Gosselain, GRIL, Departement de Sciences Biologiques, Université de Montréal, C.P. 6128, succursale Centre Ville, Montreal, QC H3C 3J7, Canada, personal communication). The resulting suspensions were filtered through 0.45-µm GF/C filters, wrapped in aluminum foil, and kept frozen for chlorophyll *a* analysis. Surface areas of the plant samples were estimated using a ruler, and biomass was calculated as chlorophyll *a* per unit area (cm^2). Phytoplankton samples (3/wetland) were filtered through 0.45-µm GF/C filters and then stored frozen in aluminum foil until analysis. Chlorophyll *a* from natural (plant) substrata and phytoplankton from the water column were extracted from frozen filters and analyzed as above. Chlorophyll *a* content for both CHL_{epi} and CHL_{peri} were expressed as µg·cm substrate sampled^{-2}, whereas values for CHL_{phyto} were expressed as µg·L water sampled^{-1}. Because the time period for colonization of periphyton was known, CHL_{peri} is expressed as a rate, standardized to 28 days (µg·cm^{-2}·28 days^{-1}).

Water samples for all nutrients were analyzed for soluble reactive phosphorus (SRP) and total phosphorus (TP) following standard methods (APHA 1992). Following digestion by potassium persulfate, TP was analyzed according to Murphy and Riley (1962) and measured on a Milton Roy 301 spectrophotometer. Total Kjeldahl nitrogen (TKN), total nitrate nitrogen (TNN), and total ammonia nitrogen (TAN) were measured with Hach protocols and reagents (Hach Company 1989) using a Hach DR2000 spectrophotometer (Hach, Loveland, Colo.). Total nitrogen (TN) was calculated by addition of TKN and TNN. Samples that had been preserved for Winkler titration in the field were processed within 4–6 h of collection, with a Hach digital titrator to

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confirm dissolved oxygen (DO) values measured in situ. Water samples for total suspended solids (TSS) determination were filtered through preweighed GF/C filters and frozen until processing. Filters were first dried at 100°C for 1 h, dried in a dessicator with calcium sulphate for another hour, and then weighed to determine TSS. Loss on ignition was determined after combustion at 550°C for 20 min followed by drying in the dessicator for an hour. Weight of the combusted filter was assumed to be total inorganic suspended solids (TISS), whereas difference in the weight of the filter before and after combustion was total organic suspended solids (TOSS).

Sediment samples for analysis of sediment total phosphorus (Sed TP), inorganic content (Sed Inorg) and exchangeable ammonia (Sed NH_4) were frozen within 12 h of collection. To determine the percentage of inorganic material in the sediment, the sample was dried at 40°C and then placed in a desiccator and weighed to determine the starting amount. This sample was then combusted at 550°C for 1 h and weighed again, and loss on ignition was calculated as Sed Inorg. Sediment phosphorus was determined using the ignition method described by Andersen (1976) and is expressed as milligrams per gram of combusted sediment. For determination of Sed NH_4, the sediment was first dried overnight at 40°C. The ammonium was then extracted from approximately 1.5 g of sediment using 25 mL of 2 M potassium chloride. This mixture was shaken vigorously for 30 s and allowed to sit overnight. On the next day, the samples were again shaken for 30 s and filtered through GF/C filters. The ammonium content of the sample was determined by addition of Nessler reagent and subsequent colorimetric analysis using the Hach DR2000 spectrophotometer. Two replicates were carried out for most sites; however, those with very high Sed NH_4 values needed to be diluted and time only permitted one replicate in these cases.

Statistical methods

Statistical analyses were performed using SAS JMP software version 3.5 for the Macintosh (SAS Institute Inc., Cary, N.C.). Data were standardized (to mean of zero and standard deviation of 1) before a principal components analysis (PCA; Digby and Kempton 1987). All environmental and algal data were $\log_{10}$-transformed to normalize the data before conducting regression and correlation analyses. Percent cover of submergent vegetation data were arcsin-transformed before analysis.

Results

Environmental gradient

Degraded wetlands had high nutrient concentrations, poor water clarity (high suspended solids, turbidity, light extinction coefficient), and high conductivity. At the other end of the gradient, pristine wetlands exhibited low nutrient levels and good water clarity. Because the wetlands were not randomly selected, the range of values are not representative of general wetland quality for the lakes in question; on the contrary, we made a deliberate effort to include a selection of high-quality and poor-quality wetlands in each Great Lake. Nevertheless, there was an obvious bias towards more degraded wetlands in the lower lakes compared with those in the upper lakes, and this tendency was also reflected in nutrient data for respective water column and sediment samples. Latitude, longitude, and variables associated with water clarity (turbidity, light extinction, TSS, TISS, TOSS) and those related to anthropogenic disturbance (conductivity, TDS; Lougheed et al. 2001) are presented, along with the nutrient data, on a lake-by-lake basis in Appendix A. It is noteworthy that wetlands with the lowest nutrient levels and clearest water were located in the Lake Superior basin, in areas characterized by relatively minimum human influence (Cloud Bay), whereas those with the highest nutrient levels and most turbid water were located in one of the most heavily impacted areas of Lake Erie, the western basin (Old Woman Creek; Lougheed et al. 2001).

A PCA was used to produce the three synthetic axes that best capture overall variation in the data set and to identify the environmental variables that contribute most to these axes. Of 21 measured environmental variables, 10 were found to be most significant in explaining the variation in the data and were included in this analysis (Table 2). The only direct measure of light availability, the light extinction coefficient, was not included in the PCA because of missing data for five wetlands. However, light extinction was strongly related to turbidity ($r^2 = 0.93$, $p < 0.0001$) in the 19 wetlands in which both were measured. Initial analysis included all 24 wetlands in the PCA, but Jordan Harbour appeared to be an outlier because of extremely high inorganic suspended solids in the water (134.4 $mg \cdot L^{-1}$ compared with 67.23 $mg \cdot L^{-1}$ at Old Woman Creek, the next highest measurement). This site is located in the highly agricultural Niagara Peninsula, and it was sampled immediately after an extremely heavy rainstorm when sediment loading from the watershed was high. We removed this site from the PCA because it did not reflect the same type of disturbance regime as the other wetlands and then reanalyzed the data including 23 wetlands. The first three principal components accounted for almost all variation in the data set (91%). PC1 accounted for 69% of the variation in the data and was highly correlated with TP and water clarity variables (turbidity, suspended solids) and more weakly correlated with conductivity, TDS, and nitrogen (TKN, TN, TAN). All of these variables are associated with degraded aquatic environments in Great Lakes wetlands (Lougheed et al. 2001; Lougheed and Chow-Fraser 2002). A further 13% was accounted for by PC2, which was primarily correlated with nitrogen variables (TN, TKN) and negatively correlated with conductivity. Another 9% was explained by PC3, which was positively correlated with TDS and negatively correlated with TISS.

The PCA biplot illustrates the distribution of coastal wetlands along the two major environmental axes (Fig. 2). Sites with high PC1 values (Old Woman Creek of Lake Erie and Cootes Paradise of Lake Ontario) had correspondingly high water turbidity and TP concentrations. Sites with low PC1 values had clear water and low TP concentrations and included all sites in the upper lakes as well as several better-quality sites in the lower lakes (e.g., Cloud Bay, Lost Creek, and West Fish Creek of Lake Superior, Sandy Creek of Lake Ontario, and Turkey Point of Lake Erie). PC2 separated wetlands along a nitrogen and conductivity axis, with Mismer

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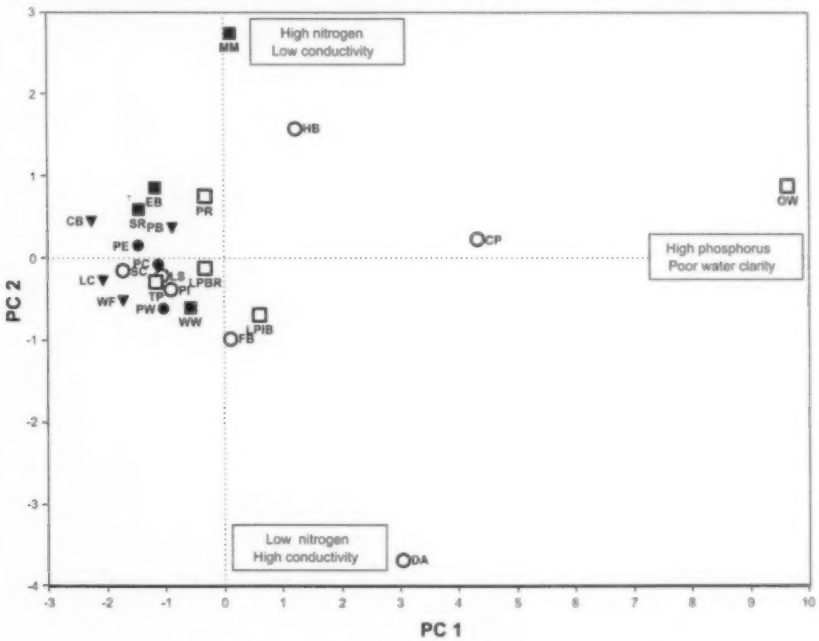
Table 2. Results of principal components analysis (PCA) of environmental data for 23 wetland sites and correlations between environmental variables and principal components axes.

Principal components axis	Variance explained, %	Environmental variable	Correlation coefficient r	p value
PC1 (eigenvalue = 6.9)	69	TP	0.95	<0.0001
		TSS	0.93	<0.0001
		TOSS	0.93	<0.0001
		Turbidity	0.91	<0.0001
		TISS	0.89	<0.0001
		Conductivity	0.76	<0.0001
		TDS	0.75	<0.0001
		TKN	0.73	0.0001
		TN	0.72	0.0001
		TAN	0.64	0.0010
PC2 (eigenvalue = 1.3)	13	TN	0.61	0.0020
		TKN	0.59	0.0028
		Conductivity	–0.50	0.0163
		TDS	–0.45	0.0292
PC3 (eigenvalue = 0.9)	9	TDS	0.44	0.0361
		TISS	–0.42	0.0484

Note: TP, total phosphorus; TSS, total suspended solids; TOSS, total organic suspended solids; TISS, total inorganic suspended solids; TDS, total dissolved solids; TKN, total Kjeldahl nitrogen; TN, total nitrogen; TAN, total ammonia nitrogen.

Fig. 2. Plot of PC1 versus PC2 scores for 24 coastal wetlands. Open symbols are wetlands in the lower lakes (Lake Erie, open squares; Lake Ontario, open circles), solid symbols indicate wetlands in the upper lakes (Lake Huron, solid squares; Lake Michigan, solid circles; Lake Superior, solid triangles). Letter codes shown in the plot for each wetland are listed in Table 1.



Marsh at one end (high nitrogen levels, average conductivity) and Darlington at the other (high conductivity, relatively low nitrogen levels).

Interpretation of how water quality in our wetlands responds to anthropogenic stressors is premised on the assumption that these variables are unaffected by large-scale regional differences relating to latitude or climate. We tested for this confounding effect by including latitude in the PCA, but found latitude to be more weakly correlated with PC1 than the other environmental variables ($r = –0.56$, $p = 0.0055$) and to have no significant correlation with the other two PC axes. Because mean August temperature was a significant

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Table 3. Mean chlorophyll *a* biomass for periphyton (CHL_{peri}) on plastic rods, epiphyton (CHL_{epi}) on submergent plants, and phytoplankton (CHL_{phyto}) at each wetland site.

Wetland code	CHL_{peri}, $\mu g \cdot cm^{-2} \cdot 28\ days^{-1}$	*n*	SD	CHL_{epi}, $\mu g \cdot cm^{-2}$	*n*	SD	CHL_{phyto}, $\mu g \cdot L^{-1}$	*n*	SD
OW	166.13	20	56.69	20.27	10	23.71	100.35	3	3.28
CP	65.24	34	39.36	72.19	9	37.84	65.37	12	22.16
FB	32.05	12	14.09	12.87	8	13.76	7.42	6	4.00
JH	23.13	19	14.39	5.34	10	4.61	2.84	3	0.00
LPIB	22.19	16	24.47	8.37	9	11.61	4.02	6	2.37
PW	20.63	20	9.65	6.28	10	6.18	2.70	3	0.62
LS	17.12	18	12.40	1.89	10	1.67	5.05	3	1.79
PE	15.82	15	12.39	8.98	10	11.84	1.89	3	1.08
DA	13.85	12	16.72	1.75	7	1.33	9.56	3	7.41
PR	11.41	15	14.39	3.86	9	3.38	9.28	3	6.02
SC	11.28	18	9.72	6.87	10	10.16	5.30	3	0.38
LC	10.33	20	6.36	10.15	4	13.22	2.25	3	0.74
MM	9.41	20	5.50	6.84	4	4.20	0.27	3	0.23
HB	8.55	21	6.82	17.12	18	17.66	13.44	6	11.28
PB	6.57	8	6.15	38.86	6	62.70	2.46	3	0.33
PC	4.83	12	3.55	1.02	6	0.78	3.98	3	1.61
LPBR	3.90	16	1.93	3.22	8	3.19	4.24	8	3.30
PI	3.15	19	2.92	1.63	9	2.27	1.28	3	0.33
TP	2.80	20	2.49	0.94	10	1.01	2.49	6	2.28
SR	2.51	18	2.22	3.27	5	0.57	4.92	3	0.87
CB	1.63	19	1.24	8.65	10	7.02	0.09	3	0.08
WW	1.49	17	2.44	4.65	10	4.92	3.98	3	0.28
EB	1.23	18	0.74	1.05	5	0.16	1.08	3	0.62
WF	0.85	18	0.55	2.95	4	2.59	0.46	3	0.42

Note: Data are ordered by decreasing periphyton biomass. SD, standard deviation.

predictor of growing season length ($r^2 > 0.95$, $p < 0.001$), we substituted mean August temperature and reran the PCA but still found no change in the outcome of the PCA (data obtained from Agriculture and Agri-Food Canada 2002; Midwest Regional Climate Center 2002). Therefore, we are confident that the results of the PCA using water-quality variables alone can be interpreted as the response of wetlands to varying degree of anthropogenic stress.

Phytoplankton, epiphyton, and periphyton biomass

We have organized the algal data for the 24 wetland complexes in descending order according to CHL_{peri} (Table 3). Incubation periods for periphyton varied from 24 to 31 days, so we have standardized the data to $\mu g \cdot cm^{-2}$ for a 28-day period to ease comparison. Although 20 rods were installed at each site (except Cootes Paradise, which had 35 rods), some were lost before collection, and between 8 and 34 samples were collected at each site (as shown in Table 3). Mean CHL_{peri} for all wetlands was 19.18 ± 7.02 (± SE) $\mu g \cdot cm^{-2} \cdot 28\ days^{-1}$, with values ranging from a low of 0.85 in relatively undisturbed West Fish Creek wetland to a high of 166.12 $\mu g \cdot cm^{-2} \cdot 28\ days^{-1}$ in the heavily disturbed Lake Erie wetland, Old Woman Creek. Because the colonization period for epiphyton could not be determined, we expressed the values simply as $\mu g \cdot cm^{-2}$. For the majority of the wetlands, ~10 epiphyton samples were obtained on two sampling occasions (sample numbers for each site are shown in Table 3). Mean CHL_{epi} was 10.34 ± 3.18 (±SE) $\mu g \cdot cm^{-2}$, with a range of 0.94 $\mu g \cdot cm^{-2}$ in Turkey Point, a high-quality Lake Erie marsh, to 72.19 $\mu g \cdot cm^{-2}$ in degraded Cootes Paradise Marsh of Lake Ontario. These values are higher than the range reported in Goldsborough and Robinson (1996) of 0–37 $\mu g \cdot cm^{-2}$ for epiphyton in freshwater marshes. Mean CHL_{phyto} was 10.61 ± 4.71 (±SE) $\mu g \cdot L^{-1}$, with values ranging from 0.09 $\mu g \cdot L^{-1}$ in pristine Cloud Bay wetland of Lake Superior to 100.35 $\mu g \cdot L^{-1}$ in heavily impacted Old Woman Creek, and fall within the range of values reported for Great Lakes wetlands by Lougheed et al. (2001).

We can compare the trends in CHL content for each algal group across the striking degradation gradient, as indicated by the PC1 site score (Fig. 3*a*). Mean CHL_{peri} decreased predictably as wetlands changed from poor-quality (Old Woman Creek and Cootes Paradise) to high-quality marshes (Lost Creek and Cloud Bay) (Fig. 3*b*). CHL_{epi} (Fig. 3*c*) and CHL_{phtyo} (Fig. 3*d*) showed similar decreases.

To test the relationship between each of the algal variables with water-quality degradation, we regressed CHL_{peri}, CHL_{phtyo} (Figs. 4*a*, 4*b*, respectively), and CHL_{epi} against PC1 site scores. Consistent with our first hypothesis, we found significant relationships between PC1 score and CHL_{peri} and CHL_{phyto}, respectively accounting for 54 and 70% of the variation in periphyton and phytoplankton data ($p < 0.001$ for both; Fig. 4). Poor-quality wetlands with high turbidity and nutrients supported high biomass of phytoplankton and rapid colonization by periphyton. By comparison, PC1 was not a significant predictor of CHL_{epi} ($r^2 = 0.19$, $p = 0.039$).

Correlations between each of the environmental variables used in the PCA and the three algal groups were performed

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Fig. 3. Values of (*a*) PC1 score and chlorophyll *a* biomass (mean ± 1 standard error) of (*b*) periphyton (CHL_{peri}), (*c*) epiphyton (CHL_{epi}), and (*d*) phytoplankton (CHL_{phyto}) for each of 24 coastal wetlands. In each plot, wetlands are ranked according to PC1 value or chlorophyll *a* biomass, decreasing from left to right.

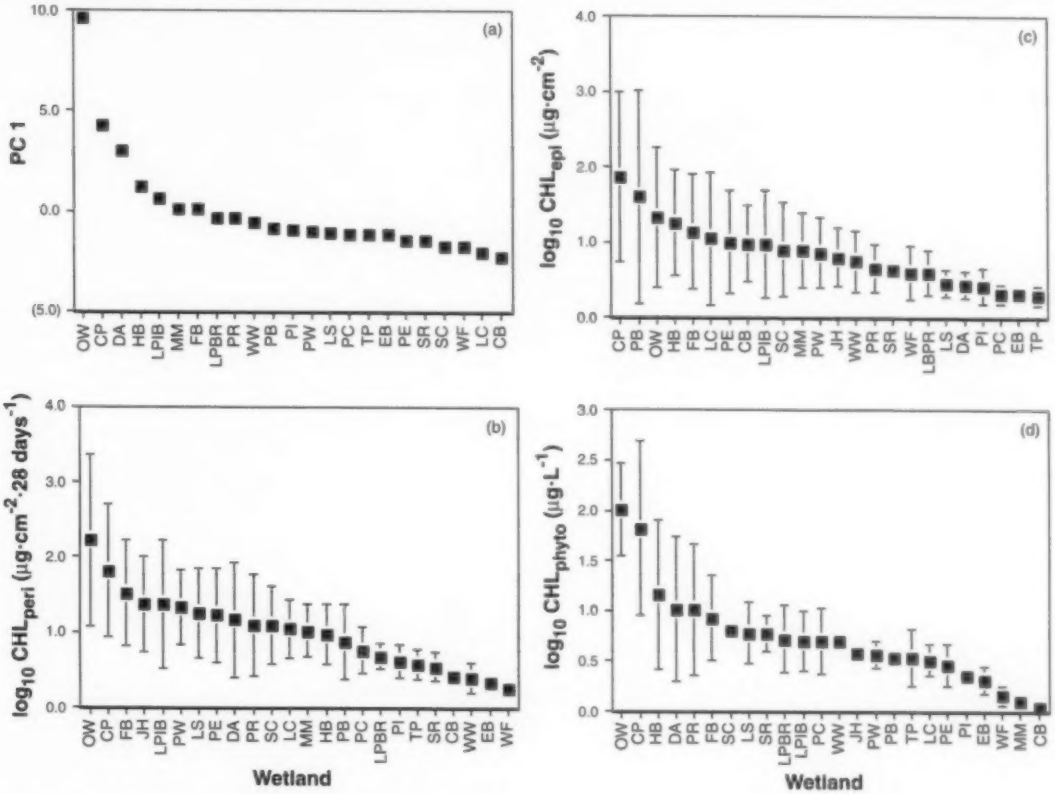
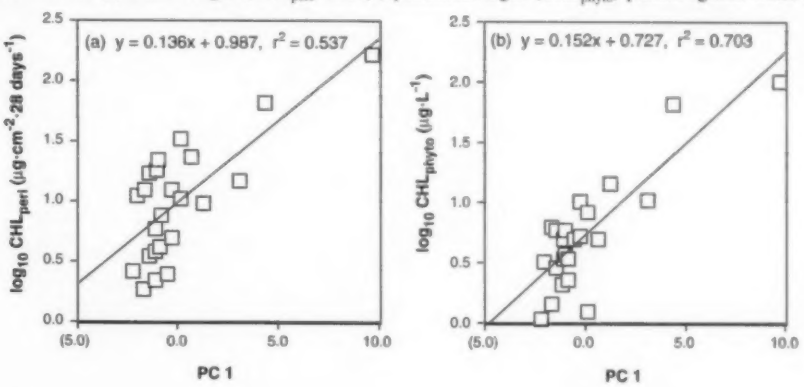


Fig. 4. Chlorophyll *a* biomass for (*a*) benthic algae (CHL_{peri}) and (*b*) planktonic algae (CHL_{phyto}) plotted against water quality (PC1 scores).



using log_{10}-transformed data (Table 4). CHL_{phyto} was significantly correlated with all environmental variables ($p < 0.05$), whereas CHL_{peri} was significantly correlated with all but TISS, and CHL_{epi} was only significantly correlated with turbidity and TAN. CHL_{peri} was positively correlated with TOSS, conductivity, TDS, and TP, suggesting that high nutrient concentrations stimulate the colonization rate of attached algae, even though light penetration of the water column is reduced. The strong relationship between CHL_{phyto} and TOSS, conductivity, TSS, TDS, TP, and nitrogen (TKN, TN, TAN) may be spurious because phytoplankton are included in the measurement of five of these parameters (TOSS, TSS, TP, TKN, and TN). Both CHL_{peri} and CHL_{phyto} were negatively correlated with latitude ($r = -0.57$, $p = 0.004$ and $r = -0.68$, $p = 0.0004$, respectively); the most northern sites in Lake Superior generally had very low algal biomass, but these

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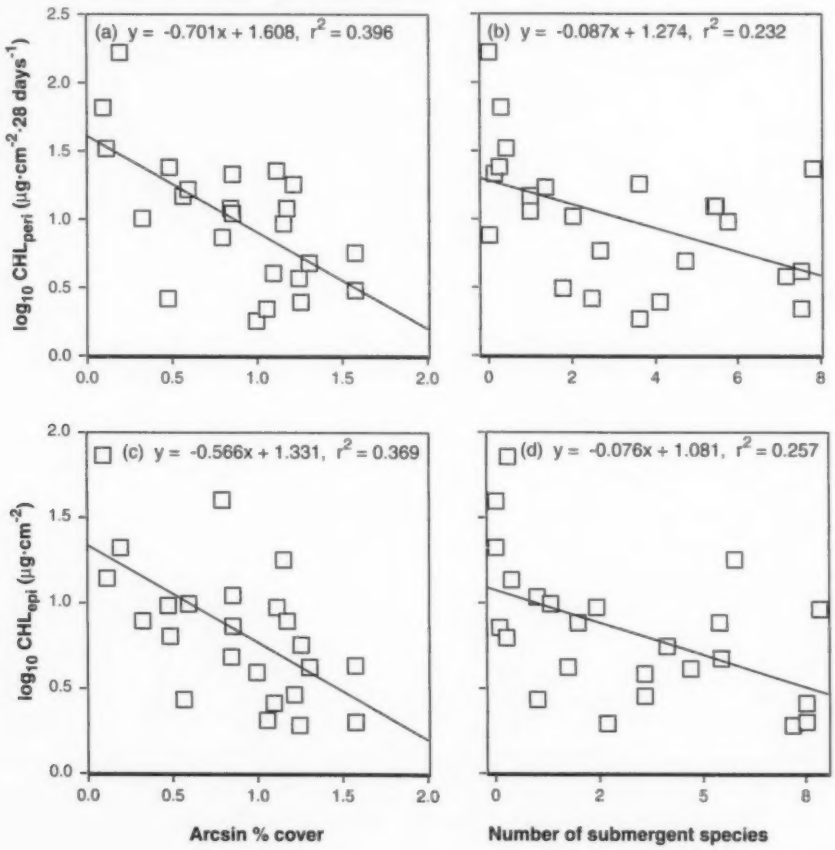
Table 4. Correlations between benthic (CHL_{peri} and CHL_{epi}) and planktonic (CHL_{phyto}) chlorophyll *a* biomass and PC1 and the environmental variables associated with PC1.

	CHL_{peri}		CHL_{phyto}		CHL_{epi}	
	r	*p* value	*r*	*p* value	*r*	*p* value
PC1	0.73	0.0001	0.84	<0.0001	0.43	0.0400
Turbidity	0.54	0.0076	0.62	0.0014	0.44	0.0349
TSS	0.53	0.0100	0.71	0.0001	0.38	0.0800
TOSS	0.68	0.0004	0.84	<0.0001	0.35	0.1005
TISS	0.34	0.1119	0.53	0.0093	0.34	0.1157
Conductivity	0.63	0.0013	0.72	0.0001	0.13	0.5650
TDS	0.62	0.0015	0.70	0.0002	0.16	0.4679
TP	0.59	0.0032	0.70	0.0002	0.24	0.2776
TKN	0.51	0.0138	0.58	0.0038	0.37	0.0826
TN	0.51	0.0122	0.56	0.0053	0.35	0.0971
TAN	0.54	0.0074	0.69	0.0003	0.64	0.0010
CHL_{peri}			0.75	<0.0001	0.58	0.0034
CHL_{phyto}	0.75	<0.0001			0.47	0.0229
CHL_{epi}	0.58	0.0034	0.47	0.0229		

Note: TSS, total suspended solids; TOSS, total organic suspended solids; TISS, total inorganic suspended solids; TDS, total dissolved solids; TP, total phosphorus; TKN, total Kjeldahl nitrogen; TN, total nitrogen; TAN, total ammonia nitrogen.

Fig. 5. Log_{10} chlorophyll *a* biomass of periphyton (CHL_{peri}) and epiphyton (CHL_{epi}) plotted against (*a* and *b*) arcsin % cover of submergent plants and (*c* and *d*) number of submergent plant species.


(a) y = -0.701x + 1.608, r² = 0.396
(b) y = -0.087x + 1.274, r² = 0.232
(c) y = -0.566x + 1.331, r² = 0.369
(d) y = -0.076x + 1.081, r² = 0.257
log10 CHLperi (µg·cm-2·28 days-1)
log10 CHLepi (µg·cm-2)
Arcsin % cover
Number of submergent species

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sites also have lower anthropogenic impacts (consequently lower nutrient and sediment levels). There was a strong positive correlation between CHL_{phyto} and CHL_{peri} ($r = 0.75$, $p < 0.0001$), whereas CHL_{epi} was more weakly correlated with CHL_{phyto} ($r = 0.47$, $p = 0.02$) and CHL_{peri} ($r = 0.58$, $p = 0.0034$).

Submergent macrophytes

Our second hypothesis predicts that both epiphyton and periphyton will be inversely related to areal cover of submersed aquatic vegetation. We regressed algal biomass against both percent cover and species richness of submergent plants (Fig. 5). CHL_{peri} and CHL_{epi} biomass decreased with percent cover of aquatic plants ($r^2 = 0.40$, $p = 0.001$ and $r^2 = 0.37$, $p = 0.0016$, respectively) and with species richness of macrophytes ($r^2 = 0.23$, $p = 0.017$ and $r^2 = 0.26$, $p = 0.0115$, respectively), whereas there was no significant correlation between CHL_{phyto} and either macrophyte variables. These results reveal that high rate of colonization by attached algae, low species richness, and low percent cover of submergents are found in degraded wetlands.

Discussion

The 24 wetlands in this study represent a very large disturbance gradient that was accurately reflected in the synthetic axis, PC1. There was a highly significant linear relationship between the water quality of wetlands and PC1 scores, and previous studies have demonstrated that variation in PC1 scores can be attributed to alteration in land uses in wetland watersheds (Crosbie and Chow-Fraser 1999; Lougheed et al. 2001; P. Chow-Fraser, unpublished data). From this study, it is clear that regional variation in climate and bedrock geology did not contribute a significant amount to the variation in water-quality characteristics of these wetlands.

That CHL_{peri} and CHL_{phyto} are both strongly related to PC1 scores bodes well for using these as indicators of wetland degradation. In undisturbed wetlands, both periphyton and phytoplankton biomass are limited by low nutrient availability, whereas in highly disturbed wetlands, excessive nutrient loading results in high CHL_{peri} and CHL_{phyto}. By comparison, the relationship between CHL_{epi} and disturbance is complicated by the fact that when nutrients become limiting in the water column, epiphytic algae may be able to obtain nutrients leaked from the vascular plants they colonize or from nutrients recycled within the epiphytic matrix of living and detrital material attached to the plant surface (Burkholder 1996; Wetzel 1996).

It is not possible to conduct a meaningful numerical comparison of all three variables because periphyton and epiphyton do not share the same colonization periods, and phytoplankton data are expressed volumetrically, whereas the other two are expressed on an areal basis. Nevertheless, the utility of each type of algae for biomonitoring can be compared in other ways. Periphyton grown on artificial substrata allow for standardization of both time and substrate characteristics, reducing the variation found in epiphyton and phytoplankton samples and providing for more meaningful site-to-site comparisons. The acrylic rods used in this study are easily installed, collected, and processed. The chief disadvantage to using this method is that two trips to each site are required. Epiphyton samples are most representative of the natural benthic algae community, but many unquantified factors such as successional stage, senescence, or seasonal internal community changes could be influencing the biomass estimates. Epiphyton samples require more processing time at collection, and chlorophyll *a* biomass estimates may be confounded by incomplete removal of algae from the vascular plant material or by inclusion of macrophyte chlorophyll *a* in the sample. In addition, estimating surface area of submergent plants is difficult and subject to greater measurement error compared with artificial substrata. Although phytoplankton samples are easily collected and processed, their spatial and temporal variability in degraded wetlands (Chow-Fraser 1999) make them difficult to sample accurately without a great deal of effort or without the use of expensive in situ probes. In this study, periphyton grown on artificial substrata provided the most precise data because of the higher degree of control and the relative ease of collecting a large number of samples.

The main external factors that regulate the biomass of algae in wetlands include light availability, primary nutrient concentrations, and grazing pressure from benthic and planktonic herbivores. Compared with benthic algae that are sessile and that obtain their nutrients from the biofilm attached to substrate (Burkholder 1996), planktonic algae obtain all of their nutrients from the water column. Hence, phytoplankton are less constrained by low light availability when there is abundant nutrient in the water column because they can move towards the light and shade out other primary producers. However, they are vulnerable to grazing because they reside in the same medium in which zooplankton grazers live (Borchardt 1996). In this regard, benthic algae may have an advantage in enriched environments, because the community of benthic invertebrates that graze on periphyton and epiphyton tend to be depressed, presumably because of the presence of a large number of benthivorous fish (Chow-Fraser 1998). Thus, even though benthic algae can be limited by light, their biomass is unconstrained by grazing pressure in degraded wetlands. In contrast, when benthic algae occur in good-quality marshes that have abundant macrophytes, benthic grazers tend to be abundant and keep the benthic algal biomass at low levels (Goldsborough and Robinson 1996).

The large site-to-site differences in the biomass of phytoplankton, epiphyton, and periphyton measured in this study are most likely due to the combined effects of nutrient limitation, light availability, and grazing pressure rather than to a single factor. The limiting factor for a particular site must be deduced experimentally. The decline of submergent macrophytes in degraded wetlands has been attributed to high epiphytic and planktonic algal biomass resulting from nutrient enrichment of freshwater ecosystems (Hough et al. 1989; Chow-Fraser 1998; Lougheed et al. 2001). The relationship between both areal cover and species richness of submergent plants found in this study is in agreement with these previous studies. There is a strong negative association between high benthic algal biomass and presence of submergent plants. Although it is not possible to state whether the benthic algae are the cause of macrophyte decline in degraded wetlands, it is clear that a suite of measurable factors are implicated in the loss of significant wetland habitats.

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We have shown that the biomass of periphyton grown on acrylic rods can be used to indicate the degree of wetland degradation relating to anthropogenic nutrient loading and concomitant alterations in water clarity. The standardized protocol that we used provides a relatively precise measure of wetland quality and is better for this purpose than phytoplankton or epiphyton growing on vascular plant material. Information regarding the taxonomic affiliation of the planktonic and benthic algae should provide further insight into wetland algal ecology and enhance the use of these organisms as bioindicators. We recommend that both planktonic and benthic algal biomass be included in routine monitoring and management of coastal wetlands.

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Appendix A

Appendix appears on the following page.

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Table A1. Location of wetland sites and measured environmental variables for 24 Great Lakes coastal wetlands included in this study.

Lake	Wetland, state or province	Wetland code	Latitude	Longitude	Turbidity (FTU)	Light extinction	Conductance ($\mu S \cdot cm^{-1}$)	TDS ($g \cdot L^{-1}$)	DO ($mg \cdot L^{-1}$)	pH	Temperature (°C)
Superior	Cloud Bay, Ont.	CB	48.0828	–89.4372	2.89	2.62	73.5	0.039	8.77	7.83	15
Superior	Pine Bay, Ont.	PB	48.0333	–89.5195	17.19	2.47	101.1	0.045	8.05	7.98	18
Superior	Lost Creek, Wis.	LC	46.8586	–91.1358	4.87	3.40	102.1	0.065	4.21	7.15	20
Superior	West Fish Creek, Wis.	WF	46.5869	–90.9458	11.03	2.06	102.6	0.066	9.30	7.72	16
Michigan	Portage Creek, Wis.	PC	45.7062	–87.0800	7.06		267.4	0.212	9.91	8.37	22
Michigan	Peshtigo Marsh, Wis.	PE	44.9840	–87.6607	2.06	3.44	247.2	0.164	7.43	8.15	25
Michigan	Pentwater, Mich.	PW	43.7628	–86.4078	4.95	2.80	383.4	0.269	10.10	8.63	22
Huron	Echo Bay, Ont.	EB	46.4944	–84.0761	16.99		84.0	0.054	8.82	8.31	23
Huron	Spanish River, Ont.	SR	46.1850	–82.3306	4.06		145.3	0.093	8.81	8.05	23
Huron	Mismer Marsh, Mich.	MM	46.0051	–84.4606	3.12		215.5	0.140	9.73	8.34	22
Huron	Wigwam Bay (Pine River), Mich.	WW	43.9702	–83.8543	4.42	2.54	425.1	0.249	10.32	9.27	24
Ontario	Hay Bay, Ont.	HB	44.1750	–76.9250	5.78	1.63	495.3	0.333	6.18	7.73	22
Ontario	Presqu'Ile, Ont.	PI	44.0154	–77.7306	4.33	2.21	298.3	0.363	9.09	8.07	23
Ontario	Darlington, Ont.	DA	43.8730	–78.7970	21.65	5.00	1171.5	0.664	6.21	8.47	21
Ontario	Frenchman's Bay, Ont.	FB	43.8123	–79.0947	30.35	2.70	426.3	0.270	8.88	8.66	25
Ontario	Sandy Creek, N.Y.	SC	43.7009	–76.1965	1.95	1.72	217.2	0.149	10.15	9.01	24
Ontario	Little Sodus, N.Y.	LS	43.3390	–76.6944	2.32	2.67	319.9	0.233	8.25	8.14	24
Ontario	Cootes Paradise, Ont.	CP	43.2800	–79.8980	42.12	6.65	676.9	0.511	10.28	8.21	25
Ontario	Jordan Harbour, Ont.	JH	43.1600	–79.3700	116.73	13.17	488.0	0.311	6.06	7.74	22
Erie	Turkey Point, Ont.	TP	42.6485	–80.3417	3.96	2.05	315.7	0.203	7.83	8.33	22
Erie	Long Pt., Big Rice Bay, Ont.	LPBR	42.5893	–80.3355	3.29	1.87	301.4	0.172	8.91	8.43	21
Erie	Long Pt., Inner Bay, Ont.	LPIB	42.5867	–80.3877	16.70		313.1	0.348	7.24	8.20	20
Erie	Presque Isle, Pa.	PR	42.1590	–80.0985	4.70	3.22	273.4	0.163	7.39	8.41	23
Erie	Old Woman Creek, Ohio	OW	41.3822	–82.5145	90.56	11.01	774.5	0.495	5.04	8.05	22

Note: Data are ordered by decreasing latitude by lake. TDS, total dissolved solids; DO, dissolved oxygen.

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Table A2. Nutrients and dissolved solids for water column and sediment samples from each wetland site.

Wetland code	TP ($\mu g \cdot L^{-1}$)	SRP ($\mu g \cdot L^{-1}$)	TKN ($mg \cdot L^{-1}$)	TNN ($mg \cdot L^{-1}$)	TAN ($mg \cdot L^{-1}$)	TN ($mg \cdot L^{-1}$)	TN:TP	TSS ($mg \cdot L^{-1}$)	TISS ($mg \cdot L^{-1}$)	TOSS ($mg \cdot L^{-1}$)	Sed TP ($mg \cdot L^{-1}$)	Sed NH_4 ($mg \cdot g^{-1}$)	Sed Org ($g \cdot g^{-1}$)	Sed Inorg ($g \cdot g^{-1}$)
CB	19.80	2.60	1.25	0.13	0.02	1.38	69.9	0.82	0.02	0.80	0.45	0.010	0.03	1.0
PB	56.26	6.25	1.25	0.27	0.10	1.52	27.0	16.88	13.68	3.20	0.47	0.023	0.08	0.9
LC	49.49	8.86	0.42	0.23	0.01	0.65	13.1	4.20	0.96	3.25	0.24	0.020	0.15	0.8
WF	59.91	10.42	0.00	0.03	0.10	0.03	0.6	9.28	6.18	3.10	0.13	0.001	0.01	1.0
PC	58.87	5.21	1.25	0.93	0.04	2.18	37.1	3.58	0.98	2.60	0.27	0.014	0.02	1.0
PE	45.84	0.52	1.67	0.30	0.02	1.97	42.9	2.08	0.00	2.08	0.10	0.001	0.01	1.0
PW	40.63	0.52	1.25	0.67	0.00	1.92	47.2	4.84	0.91	3.93	0.63	0.005	0.01	1.0
EB	76.60	4.15	1.67	0.63	0.04	2.30	30.1	6.17	4.57	1.60	0.55	0.029	0.06	0.9
SR	41.85	6.17	1.67	0.60	0.03	2.27	54.2	5.76	4.06	1.70	0.52	0.003	0.02	1.0
MM	117.14	5.16	5.00	0.57	0.07	5.57	47.5	3.64	3.37	0.27	0.52	0.175	0.11	0.9
WW	49.49	0.00	1.67	0.07	0.03	1.73	35.0	10.78	5.58	5.20	0.04	0.007	0.01	1.0
HB	133.47	6.93	5.00	0.47	0.06	5.47	41.0	9.17	5.05	4.13	0.81	0.356	0.37	0.6
PI	93.00	10.11	1.67	0.03	0.02	1.70	18.3	1.38	0.39	0.99	0.33	0.009	0.01	1.0
DA	158.36	3.65	1.25	0.10	0.01	1.35	8.5	33.12	15.52	17.60	0.91	0.165	0.20	0.8
FB	68.50	1.04	1.25	0.10	0.09	1.35	19.7	12.24	4.44	7.80	1.53	0.097	0.68	0.3
SC	44.28	1.56	1.25	0.01	0.01	1.26	28.5	2.38	0.00	2.38	0.26	0.021	0.09	0.9
LS	66.68	5.73	1.67	0.17	0.02	1.83	27.5	4.07	0.88	3.19	0.66	0.095	0.12	0.9
CP	145.60	4.85	3.95	0.53	0.75	4.48	30.8	27.95	13.78	14.17	0.71	0.038	0.07	0.9
JH	341.88	166.29	2.19	0.80	0.22	2.99	8.7	146.90	134.40	12.50	0.97	0.151	0.18	0.8
TP	41.73	2.47	1.46	0.12	0.05	1.58	37.8	5.96	2.99	2.97	0.58	0.014	0.04	1.0
LP BR	89.03	4.42	1.67	0.07	0.04	1.73	19.5	19.96	15.58	4.38	0.70	0.039	0.08	0.9
LPIB	66.42	7.29	1.46	0.85	0.05	2.31	34.8	26.51	18.45	8.06	0.56	0.011	0.02	1.0
PR	94.21	9.13	2.92	0.13	0.08	3.05	32.4	6.36	0.08	6.28	1.15	0.367	0.33	0.7
OW	398.05	14.80	6.25	0.53	0.32	6.78	17.0	91.30	67.23	24.07	0.74	0.116	0.08	0.9

Note: Site codes are the same as in Table 1A; data are ordered by decreasing latitude by lake. TP, total phosphorus; SRP, soluble reactive phosphorus; TKN, total Kjeldahl nitrogen; TNN, total nitrate nitrogen; TAN, total ammonia nitrogen; TN, total nitrogen; TSS, total suspended solids; TISS, total inorganic suspended solids; TOSS, total organic suspended solids; Sed. TP, sediment total phosphorus; Sed. NH_4, sediment ammonia; Sed. org., sediment organic content; Sed. inorg., sediment inorganic content.

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Current velocity and injection of GnRHa affect reproductive behavior and body composition of captively reared offspring of wild chinook salmon (*Oncorhynchus tshawytscha*)

B.A. Berejikian, W.T. Fairgrieve, P. Swanson, and E.P. Tezak

Abstract: Captively reared adult Pacific salmon (*Oncorhynchus* spp.) released for natural spawning exhibit deficiencies in reproductive behavior. The effects of water current velocity in rearing tanks and gonadotropin-releasing hormone analogue (GnRHa) treatment on reproductive behavior of captively reared chinook salmon (*Oncorhynchus tshawytscha*) were examined. Treatment of females with GnRHa resulted in a significantly greater degree of nest guarding, earlier (more natural) onset of spawning, and higher frequency of aggression towards males. Current velocity did not significantly affect the female reproductive behaviors examined. Males reared under high current velocities (HV) spawned for the first time 2.4 days earlier, and alpha males reared in HV tanks defended their access to spawning females better than alpha males reared in low velocity (LV) tanks. Males reared in HV tanks had 34% lower protein content at the time of death than fish reared in LV tanks. Females reared in HV tanks had 38% lower lipid content, but the effects of current velocity were nonsignificant when accounting for significant random variation among tanks within treatments. Identifying and modifying environmental conditions that control hormonal changes during final maturation may lead to further improvements in reproductive performance of captively reared salmon.

Résumé : Les saumons adultes du Pacifique (*Oncorhynchus* spp.) élevés en captivité pour servir à la reproduction naturelle font montre de déficiences dans leur comportement reproducteur. Nous avons étudié le effets de la vitesse du courant dans les bassins d'élevage et d'un traitement à un analogue de hormone de libération de la gonadotropine (GnRHa) sur le comportement reproducteur de saumons quinnat (*Oncorhynchus tsawytscha*) élevées en captivité. Le traitement de femelles à la GnRHa augmente leur comportement de surveillance du nid, enclenche la reproduction plus hâtivement (plus selon la nature) et provoque un taux plus élevé d'agression envers les mâles. La vitesse du courant n'affecte pas de façon significative les comportements reproducteurs que nous avons examinés. Les mâles élevés dans des courants rapides (HV) anticipent leur première reproduction de 2,4 jours et les mâles alpha élevés dans des bassins HV défendent leur accès aux femelles reproductrices mieux que le mâles gardés dans des bassins à courant faible (LV). Les mâles élevées dans les bassins HV ont un contenu protéinique 34 % plus faible à leur mort que les mâles des bassins LV. Les femelles élevées en bassins HV ont un contenu lipidique 38 % plus faible, mais les effets de la vitesse du courant ne sont pas significatifs si l'on tient compte de la variation aléatoire significative entre les bassins et les traitements. L'identification et la modification des conditions environnementales qui contrôlent les changements hormonaux durant la maturation finale peuvent mener à des améliorations additionnelles de la performance reproductrice de saumons élevés en captivité.

[Traduit par la Rédaction]

Introduction

Pacific salmon (*Oncorhynchus* spp.) are often reared in captivity to maturity and then released for natural spawning as a means to maintain or recover imperiled populations. Captively reared (hereafter "captive") salmon exhibit reduced reproductive performance that appears to be caused by environmental factors (Berejikian et al. 1997, 2001*a*, 2001*b*). Captive salmon are fed formulated feeds and probably expend far less energy swimming than do their free-ranging wild conspecifics. As salmon mature, energy reserves are allocated to gonadal and secondary sex characteristic development (Hendry and Berg

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B.A. Berejikian[1] **and E.P. Tezak.** National Marine Fisheries Service, Northwest Fisheries Science Center, Resource Enhancement and Utilization Technologies Division, Manchester Research Station, P.O. Box 130, Manchester, WA 98353, U.S.A.
W.T. Fairgrieve. Pacific States Marine Fisheries Commission, 45 SE 82nd Drive, Suite 100, Gladstone, OR 97027, U.S.A.
P. Swanson. National Marine Fisheries Service, Northwest Fisheries Science Center, Resource Enhancement and Utilization Technologies Division, 2725 Montlake Boulevard East, Seattle, WA 98112–2097, U.S.A.

[1]Corresponding author (e-mail: barry.berejikian@noaa.gov).

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1999) and for intrasexual competition, nest construction, nest guarding, and other reproductive behaviors. Environmental effects on reproductive behavior (Berejikian et al. 1997), morphology (e.g., Hard et al. 2000), and body composition may have associated effects on important endocrine changes (Slater et al. 1994) that occur during the final stages of maturation.

Captive coho salmon (*Oncorhynchus kisutch*) are competitively inferior to their wild counterparts: captive males lose contests for mates and participate in fewer oviposition events than wild males (Berejikian et al. 2001*a*), and wild females outcompete captive females for nest sites (Berejikian et al. 1997). Captive chinook salmon (*Oncorhynchus tshawytscha*) females frequently abandon their nests without spawning and deposit a low percentage of their eggs into the gravel; males often exhibit little courtship behavior towards nearby nest-digging females (Berejikian et al. 2001*b*). In addition, the eggs of captive chinook salmon that spawn in streams suffer high mortality. Identifying relationships between environmental conditions that salmon experience in captivity and endocrine mechanisms required for their success in natural spawning may help guide improvements in husbandry practices.

Exercise improves growth rates in numerous salmonid species, which may be caused by behavioral effects, such as reduced aggression (Adams et al. 1995) or increased feed conversion (Leon 1986). Fitness-related parameters of salmon health including fin quality, muscle hypertrophy, and swimming stamina may also be improved through moderate exercise (Davison 1997). Although exercise did not produce beneficial effects for juvenile chinook salmon (e.g., Kiessling et al. 1994), we hypothesized that general improvements in physical fitness reported for numerous salmonid species would benefit maturing chinook salmon during the physically demanding reproductive phase of their life cycle.

Gonadotropin-releasing hormone (GnRH) is the central regulator of reproduction in vertebrates, including fish. It stimulates the release of gonadotropins from the pituitary (Kobayashi et al. 1997), which in turn stimulates synthesis of gonadal sex steroids, gametogenesis, maturation, and gamete release. Injection of or implants containing GnRH analogues (GnRHa) induce ovulation and spermiation in salmonids (Mylonas et al. 1992), shorten homing duration (e.g., Kitahashi et al. 1998), and increase homing precision (A. Dittman, National Marine Fisheries Service, 2725 Montlake Blvd. East, Seattle, WA 98112, unpublished data). In the present study, water current velocity and GnRHa levels were manipulated to evaluate their independent and combined effects on reproductive performance of captive chinook salmon.

Materials and methods

Study population and rearing conditions

Eyed eggs were hydraulically sampled (Collins et al. 2000) from wild chinook salmon nests in the Dungeness River, Washington, in December 1996. The eggs were transported to the Washington Department of Fish and Wildlife (WDFW) Hurd Creek Hatchery (Sequim, Wash.) where they were incubated. The emergent fry were stocked into 1.2 m diameter circular tanks supplied with well water in February 1996. After 6 months, they were transferred (at 21 g mean mass) to 6 m diameter tanks (35.4 m^3), where rearing density did not exceed 2.3 $kg \cdot m^{-3}$. During freshwater rearing, the fish were fed a commercial semi-moist diet (BioOregon, Warrenton, Ore.). In April 1998, 240 fish were transferred to the National Marine Fisheries Service (NMFS) Manchester Research Station for rearing in seawater.

On 27 October 1999, 128 chinook salmon were injected with passive integrated transponder (PIT) tags for later individual identification and were distributed equally among four indoor tanks (diameter = 4.1 m, depth = 1 m) with two tanks each for the high velocity (HV) and low velocity (LV) treatments. Each tank received ~110 $L \cdot min^{-1}$ seawater (temperature 8.3–13.6°C; ~28‰ salinity), which was filtered (<5 μm) and ultraviolet (UV) sterilized. Flow into each tank was directed to create a circular current, and water current velocity was increased in the two HV tanks using centrifugal pumps equipped with timer-controlled variable frequency drives. Current velocities were periodically measured using a Swoffer flowmeter (Swoffer Instruments, Inc., Seattle, Wash.) at four equally spaced points along each of three concentric circles 0.3, 1.2, and 1.8 m from the tank center. Measurements were taken at planes 30 cm above the tank bottoms, and simple arithmetic means of the measurements were calculated. Between 25 January and 11 July 2000, velocities in the HV tanks at the three concentric points were 8, 40, and 56 $cm \cdot s^{-1}$ at 0.3, 1 m, and 1.8 m from the center of the tank, respectively. Velocities in the LV tanks averaged 2, 11, and 18 $cm \cdot s^{-1}$ at the respective locations. Velocities in HV and LV tanks were highest near the pump inflows. Fish in the HV tanks were acclimated to the experimental rearing conditions by gradually increasing both water current velocity and duration of exposure. Between 27 October and 12 December 1999, all groups of fish were maintained under LV conditions. Current velocities were increased in the HV tanks to about 90% of maximum for 4 $h \cdot day^{-1}$ between 13 December 1999 and 2 January 2000 and then to 16 $h \cdot day^{-1}$ until 25 January 2000, after which maximum velocities (provided above) were maintained for 16 $h \cdot day^{-1}$ until the fish were transferred to fresh water.

Fish in each tank were weighed (0.1 g), measured (1.0 mm fork length), and counted to provide growth and survival information at important points in the experiment, including 27 October 1999 (when the fish were allocated to replicate tanks), 18 February 2000 (after several months of exposure to different current velocities), 4 May 2000 (when feeding was terminated), and 11 July 2000 (transfer to fresh water). Fish were fed via timer-controlled automatic feeders with a high energy (~25% crude lipid) commercial broodstock diet (Moore-Clark Inc., Vancouver, B.C., Canada). The daily feed ration was gradually reduced from 1.3% fish biomass in October 1999 to 0.8% by the end of feeding in May 2000. There were no mortalities during the rearing period.

Between 11 and 17 July 2000, all maturing fish (identified by darkening body coloration) were transferred to two 5 m diameter tanks supplied with fresh water. A total of 73 fish matured (65 females and 8 males), including 16 and 23 fish from each of the two LV tanks and 17 and 19 from each of the two HV tanks. Most males had matured early at age-2 and age-3, which is common in chinook captive broodstocks; hence, only eight mature males were available for this study. The fish from each seawater rearing tank were divided equally

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into the freshwater holding tanks. The tanks received ~80 L·min^{-1} of recirculated water (10–12°C) from the stream channel where the reproductive behavior experiment was to be conducted. Thus, the maturing fish from both HV and LV treatments were held in common low-velocity (~10–20 cm·s^{-1}) tanks for 6–8 weeks before spawning. Thirty-three of the 65 females were trucked to the Dungeness Hatchery, located on the Dungeness River, and were spawned artificially between 3 and 23 September 2000.

On 28 August 2000, all fish to be stocked in the spawning channel were anesthetized (tricane methane sulfonate), weighed, and measured (fork length). The PIT tags were read to determine the tank of origin for each fish, and a numbered Peterson disk tag (2.5 cm) was attached for individual identification during spawning. None of the females had ovulated as of 28 August (i.e., eggs could not be expressed).

Females were released into the experimental channel (see below) on two occasions. Sixteen females (eight from each velocity treatment; four per treatment tank) that appeared to be nearest ovulation (softest bellies) were selected for the first release (release 1) so that the mean body mass of fish from the two current velocity treatments was similar. On 29 August, two of the four females from each of the four tanks (eight total) were treated with ethylene vinyl acetate co-polymer (EVAC) implant(s) containing GnRHa (Reproboost®, lots EV75-061598 and EV150-041699, purchased from Veripharm, Inc., Baltimore, Md.) by intramuscular injection. Dosages were approximately 0.075 μg·g body mass^{-1}. The remaining eight females were not implanted. Implanted fish are hereafter referred to as IMP, and nonimplanted as NIMP. Females in release 1 were stocked into the stream channel with the eight males (five LV and three HV) on 2 September 2000. None of the eight males was implanted with GnRHa. For release 2, 16 females were stocked following the same procedure as release 1 (eight females were implanted on 11 September and all 16 were stocked on 15 September 2000). The 14-day duration between releases 1 and 2 was planned so that release 1 females would be completing spawning when release 2 females were introduced, which provided approximately 15 m^2 per sexually active female from each release group.

Reproductive behavior

The spawning experiment was conducted in a 40 m × 6 m stream channel located at the NMFS Manchester Research Station (Berejikian et al. 2001*b*). Briefly, 6800 L·min^{-1} of fresh water was recirculated through the channel, with 60 L·min^{-1} flow through. Water depth ranged from 10 to 35 cm, current velocities from 0.0 to 0.6 m·s^{-1} (measured with a Swoffer velocity meter at 320 standardized locations in the channel), water temperature from 9.9 to 12.2°C, and gravel size from ~3 to 12 cm in diameter. Four floodlights positioned 4.5 m above the channel provided continuous nighttime illumination of ~4.8 lux at the water surface.

Four cameras (Watec, model 902HS purchased from Rock House Productions Inc., Middletown, NY 10941, U.S.A.) 00015 lux sensitivity @ F1.4; 26 mm lens) were positioned approximately 3.8 m above the stream channel, so that each camera captured images from 25% of the channel. Video recordings were made using time-lapse recorders (Gyyr, model TLC 2124-GY purchased from Rock House Productions Inc.) at approximately 5 frames·s^{-1} between 1700 and 0730.

Reproductive behaviors were recorded by direct observation during daylight and were categorized as nesting behavior, aggression, and spawning duration (Table 1). Each fish was observed from behind an observation blind for 10 min twice daily between 0700 and 1900. The frequencies of nest digs, covering digs, and probes (see Table 1 for definitions) were recorded. Two types of aggressive behaviors, attacks and threat displays, were recorded along with the initiator and receiver of each aggressive act (Table 1). The response variable for analysis was the sum of the behavior frequencies divided by the number of observation periods (i.e., frequency per 10 min). The proportion of all observation periods in which females were observed nest guarding (female observed biting or chasing other fish near one of her previously constructed nests) was calculated. In addition, three remote underwater cameras were used to record spawning events. When a female appeared to be preparing a new nest for spawning, a camera was positioned nearby, and the video signals were recorded in real time (JVC Super VHS, model HR S-7300-U; JVC, Cypress, CA 90630, U.S.A.).

Direct observation and overhead and underwater video data were combined to quantify the durations of three reproductive phases: (*i*) prespawning duration, (*ii*) duration of sexual activity, and (*iii*) senescence, along with the number of nests constructed by each female and the location of each nest (Table 1).

Males were evaluated for their access to nesting females and participation in spawning events. Males that dominated access to an ovipositing female were considered to have attained "alpha" status and were first to enter the nest and release milt during a particular oviposition event. Males that "sneaked" into the nest and released milt during oviposition were considered to be "beta" males for a particular oviposition event.

Egg deposition estimates

Egg deposition was estimated by subtracting the number of eggs counted in the body cavity at death from the estimated fecundity. Fecundity of fish spawning in the channel was estimated from the following mass–fecundity regression equation (fecundity = 0.852·mass (g) + 545.4; $R^2 = 0.72$, $n = 29$, $P < 0.001$) derived from cohorts reared in the same environments and spawned artificially at the Dungeness Hatchery. Fecundity data from LV- and HV-reared females were combined to establish the mass–fecundity regression for the purpose of estimating egg deposition because neither the slopes of the mass–fecundity relationships nor mean fecundity differed between LV- and HV-reared females (covariate interaction: $F_{1,25} = 0.048$, $P = 0.83$; mean fecundity: $F_{1,25} = 0.019$, $P = 0.89$).

Juvenile production

Naturally spawned eggs were left to incubate in the stream channel until ~1300 temperature units (degrees (°C) × days) had accumulated since the last female completed spawning. Fry were removed from the channel by thorough seining and electroshocking. The channel was slowly drained, and any fry remaining in small isolated pools were removed with dip nets to ensure that all fry were collected.

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Table 1. Definitions of behavioral frequencies, states, and durations measured from chinook salmon spawning in the stream channel at Manchester, Washington, in 2000.

Class	Behavior	Definition
Nesting behavior (frequency)	Probe	Female arches back and lowers anal fin into substrate in a developing nest
	Nest dig	Excavating dig performed during nest construction
	Cover dig	Dig which either covers recently deposited eggs or covers a previously constructed nest
Aggression (frequency)	To female	Total aggressive acts (threat displays + attacks) initiated by fish being observed towards other females
	From female	Total aggressive acts (threat displays + attacks) received from other females against fish being observed
	To male	Total aggressive acts (threat displays + attacks) initiated by fish being observed towards males
	From male	Total aggressive acts (threat displays + attacks) received from males against fish being observed
Duration	Prespawning	Time between introduction to the channel and first spawning event
	Sexually active	Time between first and last spawning event
	Postspawning	Time between last spawning event and death
	Postrelease	Time between introduction to the channel and death

Body composition

Whole-body proximate composition analyses were performed on all fish from the stream channel (32 females and 8 males). These fish were removed from the channel either when they were near death (i.e., they could no longer maintain an upright swimming posture and were drifting downstream) or when they died overnight and were collected the following morning. An additional 29 females were spawned artificially within a week of ovulation between 19 September and 10 October 2000. All eggs were removed from naturally spawning and artificially spawned females before the individual carcasses were placed in an individual plastic bag and were frozen (–20°C) until analyzed. Testes were largely depleted and were not removed for carcass composition.

Individual fish were prepared for analysis by repeatedly grinding the whole, partially thawed carcasses in a silent cutter (Hobart Corp. (www.hobartcorp.com)) until a smooth homogenate was obtained. Approximately 100 g of each wet sample was dried to a constant weight and stored in sealed glass vials at –20°C. Methods described in Association of Official Analytical Chemists (AOAC) (1995) were followed for proximate analysis of samples: moisture by oven drying to constant mass (16 h) at 105°C, protein (N·6.25) by nitrogen determination using a LECO FP 428 analyzer, crude lipid by Soxhlet extraction with dichloromethane, and total ash by combustion at 550°C for 16 h.

Statistical analyses

Before analyzing the effects of current velocity, hormone, and release group on female reproductive behavior, a one-way nested analysis of variance (ANOVA, tanks nested within current velocity) was performed to determine whether individual seawater rearing tanks affected any of the dependent variables. Where nested tank effects were nonsignificant, three-way ANOVAs were performed for each dependent variable. Main effects (all fixed factors) were current velocity (HV and LV), hormone (IMP and NIMP), and release group (1 and 2). Interaction terms were included in the ANOVAs when testing for main effects. Variables that did not meet ANOVA assumptions of normality and homogeneity of variance were transformed by either logarithmic or arcsine transformations. Simple correlation coefficients were calculated to identify autocorrelated dependent variables. The treatment of correlated dependent variables is described in the results section. A Dunn–Šidák procedure (Sokal and Rohlf 1996) was performed on the univariate ANOVAs to an overall error rate of $\alpha = 0.05$ for behavioral tests. Dunn–Šidák corrections were made within each behavior category (listed in Table 1): nesting behaviors, aggressive frequencies, breeding durations.

A retrospective power analysis was conducted to determine the minimum difference (i.e., the effect size) between main effect means that could be detected for each response variable at $\alpha = 0.05$ and $1 - \beta = 0.80$ (Sokal and Rohlf 1996). The mean squared error from each three-way ANOVA was used as the within-group error variance, and the noncentrality parameter was estimated from Rohlf and Sokal (1995, table GG). Where provided, observed relative differences between levels of a factor were calculated as the difference divided by the grand mean.

The effects of current velocity on time to onset of male spawning and number of participations in spawning were analyzed by two-sample t tests. A 2×2 contingency table analysis was conducted to determine whether the number of beta male spawning participations was independent of the rearing treatment experienced by the alpha male.

To determine the effects of current velocity and method of spawning (natural or artificial), proximate composition dependent variables (percents lipid, ash, and protein) and growth data were analyzed by nested two-way ANOVA with current velocity (HV or LV) and method of spawning as the main effects. Individual seawater rearing tanks were nested within the main effect of current velocity. The effect of GnRHa implants was analyzed by one-way ANOVA without testing for tank effects or method of spawning because GnRHa treatment was not applied to artificially spawned females and was applied after females had been removed from the seawater rearing tanks.

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The sample sizes for some our statistical tests (two- and three-way interactions) are quite small, and the power ($1-\beta$) to detect a given effect size will generally be low but will vary depending on the variance. Therefore, we exercise caution when drawing conclusions based on nonsignificant statistical tests. In general, if the observed effect size is small, we conclude that the treatment really had no effect on the response variable. A large observed effect size coupled with failure to reject the null hypothesis indicates that our experiment was not powerful enough to detect what may be a real effect.

Results

Behavior of chinook salmon during rearing

Fish in LV and HV rearing tanks distributed themselves in the lower two-thirds of the water column and generally remained within 1 m of the outer wall of the tanks. However, the vertical distribution of fish changed depending on light level. Fish observed in early morning were closer to the surface and then moved deeper as light intensity increased. Fish in the HV tanks were more densely aggregated, with the fish frequently changing places and apparently "drafting" off one another. Fish in both treatments seemed to avoid the low velocity area in the center of the tank. Thus, current velocities at the 1.2-m contour probably best reflect the velocities experienced by fish in both treatments.

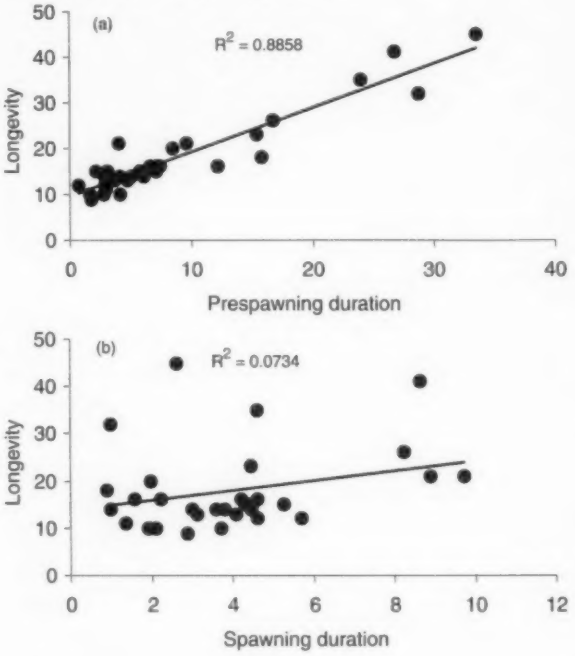
Reproductive behavior

Postrelease life span was positively correlated ($P < 0.001$) with prespawning duration; 82% of the variability in postrelease (i.e., reproductive) life span was attributable to prespawning duration (Fig. 1). Once a female had spawned the first time, time to death was fairly constant (95% confidence intervals = 8.2 days – 10.3 days, $n = 32$). The frequencies of two nesting behaviors, nest digs and probes, were significantly correlated ($r = 0.67$, $P = 0.005$) and were summed into a single dependent variable, "nest building" frequency; females often alternated between probing and digging during nest construction.

Rearing tanks, nested within the main effect of current velocity, had no significant effect on any of the variables measured ($P > 0.05$ for all tests). There were no significant two- or three-way interactions among the main effects of hormone, current velocity, and release group on any of the dependent variables ($P > 0.05$ for all tests). The effect sizes necessary to detect significant interactions shown in Table 2 indicate that the experiment had low power to detect significant differences for the interaction terms in the ANOVA model. For example, in most cases, the estimated effect size (Table 2) was as great or greater than the grand mean for a given response variable. Therefore, we cannot conclude that there were no interactions, but because none was detected, we proceeded with analysis of the main effects.

The 32 females placed in the spawning channel participated in a total of 161 spawning events (mean = 5.1, range = 1 to 8). None of the hormone treatment ($F_{1,24} = 0.03$, $P = 0.99$), current velocity ($F_{1,24} = 2.79$, $P = 0.108$), or release group ($F_{1,24} = 1.24$, $P = 0.276$) significantly affected the number of spawning events. The highest mean percent egg deposition was in the HV–IMP group (99.6%), and the lowest in the LV–NIMP group (78.7%; Table 2). However, egg deposition was not significantly affected by the main effects of current velocity ($F_{1,24} = 1.96$, $P = 0.175$), hormone ($F_{1,24} = 2.187$, $P = 0.152$), or release group ($F_{1,24} = 1.347$, $P = 0.257$). The experiment had the estimated power to detect a 38% difference in the number of spawning events between groups and a 22% difference in egg deposition. Observed main effect differences in number of spawning events ranged from 0.0 (hormone) to 22.6% (current velocity), and differences in egg deposition ranged from 8.8 (release group) to 13.3% (hormone) (Table 2). Thus, there were no main effects on egg deposition, and the power to detect differences in the number of spawning events for the main effect of current velocity was insufficient to draw a conclusion.

Fig. 1. Relationships (*a*) between female longevity (time from introduction to the stream channel to death) and prespawning duration (time from introduction to the channel to first spawning event) and (*b*) between longevity and spawning duration (time between first and last spawning event).



An estimated 118 090 eggs were deposited, and 7060 emergent fry were recovered by seining and electroshocking. Thus, only 6.0% of the eggs deposited produced free-swimming fry.

Females treated with GnRHa (IMP) took significantly less time to construct and spawn in their first nest than did NIMP females (Fig. 2*a*). Although not compared statistically, the spawn timing of IMP females resembled that of naturally spawning wild fish in the Dungeness River more closely than did the spawn timing of NIMP females (Fig. 3). Females treated with GnRHa were observed to be nest guarding significantly more often than NIMP females (Table 3; Fig. 2*b*). Females treated with GnRHa also exhibited greater levels of aggression towards males than did NIMP females (Table 3; Fig. 2*c*). Consistent with the directional effect of GnRHa implants, high current velocities resulted in a reduc-

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Table 2. The mean (± standard deviation) values for each response variable are shown for the two levels of each fixed main effect included in the three-way analyses of variance (ANOVA).

		GnRHa		Current velocity		Release group		Effect size		
Class	Dependent variable	Implanted	Nonimplanted	High	Low	First	Second	Main	2-way	3-way
Nest construction	Nest building	0.66 (0.55)	0.72 (0.68)	0.72 (0.65)	0.67 (0.58)	0.87 (0.73)	0.52 (0.42)	0.61	0.86	1.21
	Nest covering	0.76 (0.49)	0.60 (0.44)	0.82 (0.50)	0.54 (0.38)	0.75 (0.48)	0.60 (0.43)	0.52	0.73	1.04
Aggression	To female	2.10 (1.21)	1.25 (0.84)	1.84 (1.14)	1.51 (1.09)	1.48 (0.97)	1.87 (1.24)	1.13	1.63	2.31
	From female	1.97 (1.19)	1.37 (0.93)	1.59 (0.92)	1.75 (1.26)	1.55 (1.03)	1.79 (1.17)	1.17	1.68	2.38
	To male	0.60 (0.65)	0.10 (0.09)	0.40 (0.62)	0.30 (0.42)	0.50 (0.67)	0.19 (0.26)	0.47	0.68	0.96
	From male	0.44 (0.54)	0.24 (0.27)	0.20 (0.21)	0.48 (0.55)	0.39 (0.28)	0.29 (0.55)	1.34	1.93	2.73
Status	Nest guarding	0.39 (0.20)	0.15 (0.16)	0.32 (0.24)	0.23 (0.19)	0.25 (0.25)	0.29 (0.21)	0.20	0.28	0.40
Duration	Prespawning	4.62 (2.76)	12.9 (10.4)	8.22 (8.46)	9.35 (8.98)	10.86 (10.1)	6.71 (6.47)	8.20	11.82	16.71
	Active	3.48 (2.00)	4.50 (2.53)	4.29 (2.25)	3.69 (2.39)	3.81 (2.21)	4.16 (2.44)	2.49	3.55	5.07
	Senescence	5.63 (1.90)	5.18 (2.78)	5.01 (2.08)	5.81 (2.60)	5.81 (2.65)	5.00 (2.01)	2.48	3.58	5.06
Production	Number of nests	5.12 (1.59)	5.12 (2.18)	5.69 (1.54)	4.56 (2.06)	4.75 (2.02)	5.50 (1.70)	1.94	2.8	3.95
	Egg deposition	0.96 (0.07)	0.84 (0.29)	0.95 (0.14)	0.86 (0.27)	0.94 (0.27)	0.86 (0.14)	0.20	0.29	0.41

Note: The effect size was calculated as the difference between levels of each factor that could be detected at $\alpha = 0.05$ and $1-\beta = 0.80$. Effect sizes are shown for main effects (main), two-way interactions (2-way), and three-way interactions (3-way). Results for main effects of the three-way ANOVAs are shown in Table 3.

tion in time to first spawning (12.9% relative difference), an increase in nest-guarding frequency (32.7% relative difference), and an increase in aggression towards males (28.6% relative difference). However, significant differences were not detected for the main effect of current velocity (Table 3). A conclusion that current velocity had no effect is not warranted given the large estimated effect sizes (Table 2), although any potential effect is probably less than that of GnRHa.

The number of spawning participations by individual males ranged between 5 and 51. Males reared in HV tanks averaged 21.0 participations as the alpha male compared with 18.4 (18.8% relative difference) for LV-reared males (t = 0.21, 4 df, P = 0.85). Males from HV tanks averaged 3.7 participations as the beta male compared with 3.4 for LV-reared males (8.5% relative difference; t = 0.11, 4 df, P = 0.92). Significant effects may not have been detectable because of the small number of males included in the study. The proportion of spawning events in which beta males entered the nest and ejaculated at the time of spawning was significantly greater when LV-reared males were dominant (28.2%) than when HV-reared males were dominant (1.8%; χ^2 = 5.24, 1 df, P = 0.023). In other words, HV-reared males more completely monopolized access to spawning females. Males reared in the HV tanks participated in their first spawning event sooner (2.9 days) than did LV-reared males (5.3 days: t = 2.94, 4 df, P = 0.041).

Growth and body composition

Between October 1999 and May 2000, fish in both current velocity treatments grew at similar rates, increasing 61% in mass ($F_{1,54}$ = 0.90, P = 0.34) and 18% in length ($F_{1,54}$ = 0.82, p = 0.37). During the final 5 weeks of rearing in seawater, fish in both groups decreased in body mass by about 10% and increased in body length by about 3%. There were no significant differences in either length ($F_{1,54}$ = 0.51, p = 0.47) or mass ($F_{1,54}$ = 0.57, P = 0.45) at the end of the seawater rearing phase. Percent lipid was significantly affected by tanks within treatments (nested effect: $F_{2,54}$ = 5.309, P = 0.008). After accounting for the variation among tanks within treatments, current velocity had no significant effect on lipid content ($F_{1,2}$ = 2.01, P > 0.50; Fig. 4*a*). There were no significant nested tank effects on percent ash ($F_{2,54}$ = 0.312, P = 0.734) or protein ($F_{2,54}$ = 1.425, P = 0.249), and current velocity did not significantly affect ash ($F_{1,54}$ = 0.218, P = 0.642) or protein content ($F_{1,54}$ = 0.981, P = 0.326) of females (Fig. 4*a*). Females that spawned naturally in the channel had a lower percentage of whole body lipid ($F_{1,54}$ = 15.905, P < 0.001) and protein ($F_{1,54}$ = 97.849, P < 0.001) but not ash ($F_{1,54}$ = 3.634, P = 0.062) than those artificially spawned at the Dungeness hatchery (Fig. 4*c*). There were no statistically significant interactions between method of spawning and current velocity (P > 0.05 for all tests). Treatment with GnRHa had no significant effect on protein ($F_{1,30}$ = 0.340, P = 0.075), lipid ($F_{1,30}$ = 3.48, P = 0.074), or ash ($F_{1,30}$ = 0.03, P = 0.874) content of females that spawned naturally.

Males reared in HV tanks had nearly 10-fold reduction in whole body lipid content compared with males reared in LV tanks, but the difference was not statistically significant (t = −1.74, 6 df, P = 0.130; Fig. 4*b*). Males reared in HV tanks

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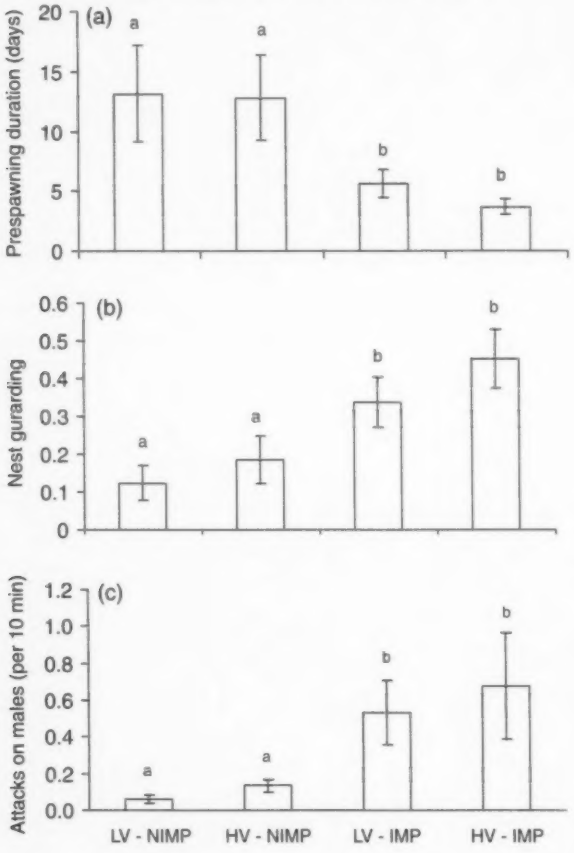
Fig. 2. Reproductive behaviors that were significantly ($P < 0.05$) affected by gonadotropin-releasing hormone analogue (GnRHa) implants are denoted by different letters. Graphs show cell means (± standard error, $n = 8$) for both levels of GnRHa (implanted (IMP) and nonimplanted (NIMP)) and both levels of current velocity (high velocity (HV) and low velocity (LV)).

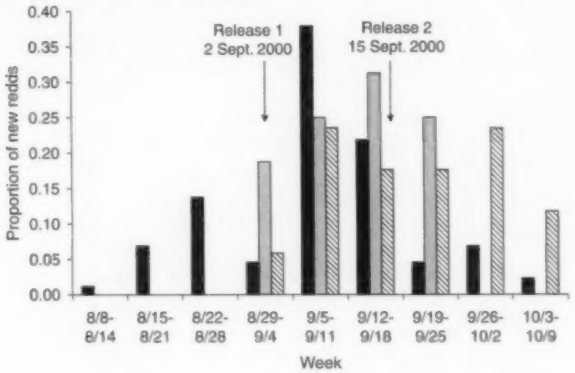


had a significantly lower percent protein content compared with LV-reared males ($t = -2.496$, 6 df, $P = 0.046$); no difference was detected in ash content ($t = 0.34$, 6 df, $P = 0.75$; Fig. 4*b*).

Discussion

GnRHa-treated females displayed a greater degree of nest guarding, earlier onset of spawning, and greater aggression towards males. Current velocity had no statistically significant effects on any female reproductive behaviors measured. Males reared in HV tanks spawned earlier than those reared in LV tanks, and alpha males reared in HV tanks better defended their access to spawning females compared with alpha males reared in LV tanks. However, the results related to male defense of females should be considered preliminary because the observations are summed for each male and are therefore not independent and because of the small number of males available in the study. Rearing in HV tanks caused

Fig. 3. Temporal pattern of new redd construction by wild and returning hatchery-reared fish in the Dungeness River (solid bars), and implanted (shaded bars), and nonimplanted (hatched bars) captive fish in the stream channel. Captive fish were released into the stream channel on 2 September (16 fish) and 15 September (16 fish) 2000. Data from the Dungeness River are from Marlowe et al. (2001). Bars represent the proportion of all redds that were newly constructed by each week. (Dates are presented in month/day format.)



males to have lower percent protein content at the time of death.

These results are the first to demonstrate effects of GnRHa implants on spawning behavior in salmon. The effects of GnRH on spawning behavior could stem directly from effects on the central nervous system or from induction of pituitary gonadotropin release and subsequent increases in peripheral levels of sex steroids. The effects of GnRH on pituitary gonadotropin production and release are well established in a wide variety of vertebrates including fish. However, there are limited data on the specific function of GnRH as a neuromodulator and (or) neurotransmitter to control reproductive behavior even though the possible role of GnRH in behavior has long been recognized (Mauk et al. 1980). For example, GnRH has been shown to control the threshold for initiation of nest-building behavior in the dwarf gourami (Yamamoto et al. 1997), to enhance courtship behavior in sparrows (Maney et al. 1997), and to alter the sensitivity of the olfactory system to pheromones in the mudpuppy (Eisthen et al. 2000). Several studies have correlated brain GnRH expression to various social behaviors in the cichlid (cf. Bushnik and Femald 1995). In salmon, GnRH expression in the olfactory nerve increases during the spawning migration (Parhar et al. 1994), and treatment with GnRHa implants enhanced homing migration (Kitahashi et al. 1998). Thus, GnRH could be involved in mediating a variety of behaviors associated with reproduction in salmon, and deficiencies in production of GnRH in the central nervous system could lead to the reproductive dysfunctions that have been observed in captive animals.

Treatment with GnRHa implants had clear effects on the reproductive behaviors of females. Although the behavior of captive fish was not directly compared with that of wild fish in our study, at least two of the three behaviors affected would likely improve reproductive performance under natural conditions. First, nest guarding is a form of parental care

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Table 3. Results of the three-way analysis of variance to determine the effects of hormone, current velocity, and release group on reproductive behavior.

		GnRHa		Current velocity		Release group	
Class	Dependent variable	*F*	*P*	*F*	*P*	*F*	*P*
Nest construction	Nest building	0.052	0.822	0.128	0.724	1.075	0.310
	Nest covering	0.957	0.337	2.552	0.123	0.881	0.357
Aggression	To female	4.829	0.037	0.589	0.450	0.776	0.387
	From female	2.385	0.135	0.067	0.798	0.469	0.500
	To male	15.473	0.001*	0.511	0.481	4.698	0.040
	From male	1.815	0.190	4.024	0.056	1.630	0.213
Status	Nest guarding	13.151	0.001*	0.181	0.674	0.064	0.802
Duration	Prespawning	8.864	0.006*	0.240	0.629	2.488	0.127
	Active	0.916	0.348	0.820	0.374	0.157	0.695
	Senescence	0.282	0.600	0.861	0.363	0.877	0.358

Note: There were no significant interactions among the three main effects for any of the dependent variables. An asterisk (*) indicates significance of Dunn–Šidák adjusted probability (*P*) values.

that guards against nest superimposition by other females. Superimposition of a female's nest may reduce offspring survival to the fry stage (Hayes 1987). Physical shock before the eyed stage of development, which under laboratory conditions has been shown to kill developing embryos (Jensen and Alderdice 1989), may be a major cause of mortality associated with superimposition. GnRHa-implanted females were observed guarding their nests more than twice as often as nonimplanted females, presumably reducing the likelihood that their nests were superimposed. Once a female has completed egg deposition, there are no costs (e.g., injury) of nest defense to future reproduction. There was no indication that the greater nest-guarding activity was associated with either a decrease in nest-guarding duration or an earlier time of death. More active nest guarding should, therefore, improve reproductive success through increased survival of the progeny unless trade-offs occur between nest guarding and other behaviors during the process of nest construction, courtship, and egg deposition.

Second, the earlier onset of spawning exhibited by GnRHa-treated females is consistent with previous studies documenting advancement and synchronization of spawn timing in artificially spawned salmon from captive broodstocks (e.g., Slater et al. 1994). The onset of spawning of IMP females created a temporal profile similar to that of wild fish spawning in the Dungeness River and should provide an advantage to the offspring under natural conditions. Salmon populations have locally adapted spawn timing (e.g., Brannon 1987), which increases the chances that offspring will emerge from the gravel under favorable environmental conditions (e.g., temperature, food availability). For example, Einum and Fleming (2000) demonstrated selective mortality on Atlantic salmon fry produced from eggs in which fertilization was artificially delayed by 10–12 days. The range of accumulated temperature units (days × °C) required for embryonic development is narrow within populations of chinook salmon (Billard and Jensen 1996). Thus, delayed spawning of captive chinook salmon (released as adults) during a declining temperature regime further delays emergence timing, may affect the condition of emerging fry (Beer and Anderson 2001), and magnifies the disparity between emergence timing of their offspring and that to which the population is locally adapted.

The effects of higher levels of aggression towards males on breeding success are more difficult to interpret than those for the other behavioral traits. Aggression towards males may represent a form of female mate selection (Maekawa et al. 1994), which in this study might suggest that IMP females were more discriminating than NIMP females in their choice of mates. Alternatively, elevated hormone levels may have created unnaturally high levels of aggression, which may interrupt nest construction and courtship, although there were no apparent delays in spawning duration of IMP females concomitant with their higher levels of aggression toward males.

The lack of significant effects of exercise on reproductive behavior follows other studies that have failed to show beneficial effects of exercise in nonmaturing chinook salmon (e.g., Kiessling et al. 1994). There are numerous possible reasons why no effect, positive or negative, was demonstrated in this study. First, the current velocities may have been inadequate to alter the parameters measured. Fish in the HV treatment experienced average current velocities of about 0.5–0.8 body lengths per second (blps) depending on their size and location in the tank. Several studies demonstrating physiological effects of sustained exercise at moderate to high swimming speeds (>1 blps) have failed to show effects at low (<1 blps) swimming speeds (e.g., McDonald et al. 1998). Second, the high velocity treatment was discontinued during the final 1.5-month freshwater holding period. For wild fish, upstream migration and holding in the river before spawning may require considerable energy expenditure depending on the distance and geomorphic properties of the river being ascended (Rand and Hinch 1998). Greater effects of exercise may be realized if the exposure to high velocity environments is continued well past the termination of feeding, perhaps to the point of reproduction. Third, females in the "control" group (i.e., NIMP–LV) exhibited a much

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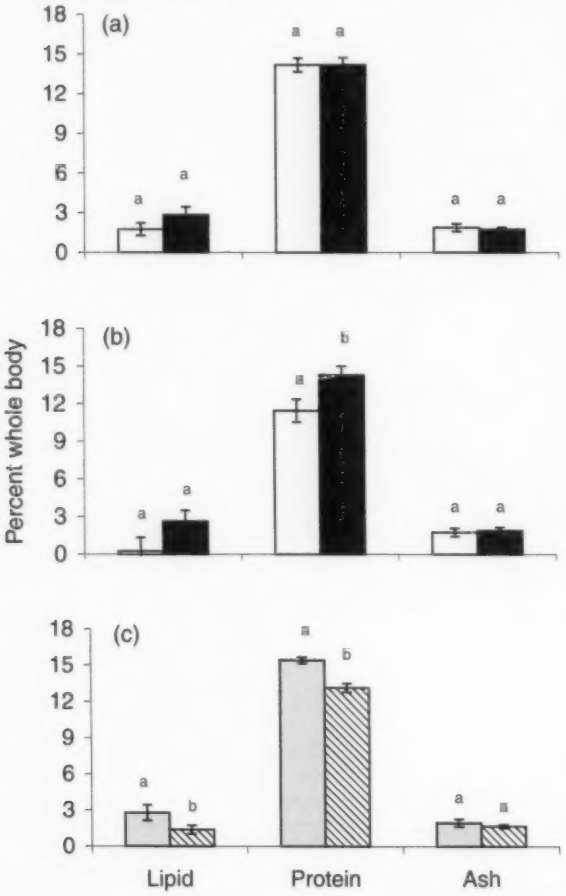
Fig. 4. Mean (± standard deviation) percentage of whole body (less gonads) lipid, protein, and ash for (*a*) females reared in high velocity (open bars, $n = 16$) vs. low velocity (solid bars, $n = 16$), (*b*) males reared in high velocity tanks (open bars, $n = 3$) vs. low velocity tanks (solid bars, $n = 5$), and (*c*) females spawned artificially (shaded bars, $n = 28$) or spawned naturally in the stream channel (hatched bars, $n = 32$). Within comparisons for protein, ash, and lipid, significant between-treatment differences are denoted by different letters ($P < 0.05$).



greater ability to deposit eggs than in previous studies of captive chinook salmon (Venditti et al. 2002), including the Dungeness River population in the same stream channel used in the present study (Berejikian et al. 2001*b*). Thus, the control treatment may have provided more favorable environmental conditions than those experienced by fish in previous studies, improving their performance and leaving little opportunity for hormone and HV treatments to improve egg deposition and associated behavioral traits.

Lack of statistical power to detect biologically significant differences may also have limited our ability to identify current velocity effects on reproductive behavior. Frequencies of nest construction and aggressive behaviors would have to differ by values about equal to the control group means to reject the null hypothesis. For example, nest-covering frequencies for the LV treatment averaged 0.54 per observation period, and the minimum detectable difference with 80% power was estimated to be 0.52. However, other response variables, including nest guarding, spawning durations, number of nests constructed, and egg deposition, had smaller estimated effect sizes that were about half of the mean values, and several that were found to be significantly different for the main effect of GnRHa were not significant for the current velocity or release group. The estimates of effect size were larger (power was lower) for two- ($n = 8$) and three-way ($n = 4$) interactions because of the smaller sample size of the interaction terms, and therefore we cannot conclude that interactions among the main effects did not exist.

Exercise caused males to have reduced protein stores at death. Compared with females that spawned artificially, females that spawned naturally, regardless of their rearing history, had lower lipid and protein levels. Hendry and Berg (1999) found that in sockeye salmon most protein loss increases during spawning. In that respect, our results support their conclusions. We could not determine whether the lower protein levels in HV males and females at death were a result of differences in their initial energy stores or a result of different energy use patterns during spawning, because samples were not collected over the course of the maturation period.

Deficiencies in the natural reproductive performance of captively reared salmon have unknown and possibly multiple causes. In this study, GnRHa injections affected several reproductive behaviors likely to improve performance. Exercise did affect body composition, measured at the time of death, in males and females and was associated with an improved ability of males to monopolize females. Identifying and modifying environmental conditions, possibly including current velocity, that control hormonal changes during final maturation may lead to further improvements in reproductive performance of captively reared salmon.

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Functional link between foraging performance, functional morphology, and diet shift in roach (*Rutilus rutilus*)

Joakim Hjelm, Gerco H. van de Weerd, and Ferdinand A. Sibbing

Abstract: This study explores how feeding performance (size-dependent attack rate) and the diet shift from zooplankton to benthic macroinvertebrates in the fish species roach (*Rutilus rutilus* L.) is related to changes in functional morphology over ontogeny. The morphological changes included overall body shape and structures of the feeding apparatus. The foraging performance of roach on zooplankton, as a function of size, was hump-shaped with a maximum of ~160 mm, and the diet shift took place around 150 mm. Over ontogeny, roach body shape gradually changed from fusiform into high-bodied. The second principal component (PC2) of the feeding apparatus had a U-shaped relationship with body size, with a minimum at 140-mm total length, which is the inverse of that found for the foraging performance on zooplankton. We suggest that changes of the feeding apparatus affect prey intake during the early zooplanktivorous phases and at the final benthivorous feeding stage, whereas prey retention ability limits the maximum intake rate at a later phase of the zooplanktivorous feeding stage. Our results also suggest the presence of both positive and negative correlations in morphology between the feeding niches, which point to the occurrence of morphological trade-offs within ontogenetic niches.

Résumé : Notre travail examine comment la performance alimentaire (le taux d'attaque relié à la taille) et le changement de régime alimentaire (du zooplancton aux macroinvertébrés benthiques) chez le gardon (*Rutilus rutilus* L.) sont reliés à des modifications de la morphologie fonctionnelle au cours de l'ontogenèse. Le changements affectent la forme générale du corps et les structures de l'appareil alimentaire. La performance de quête de nourriture du gardon dans le zooplancton en fonction de la taille suit une courbe en bosse avec un maximum près de 160 mm et il se produit un changement de régime alimentaire vers 150 mm. Au cours de l'ontogenèse, la forme du corps du gardon change graduellement de fusiforme à un corps plus haut. La deuxième composante principale (PC2) de l'appareil alimentaire montre une relation en U avec la taille du corps, avec un minimum à une longueur totale de 140 mm, ce qui correspond à l'inverse de la relation observée dans la performance alimentaire sur le zooplancton. Nous pensons que les changements de l'appareil alimentaire affectent l'ingestion des proies durant les premières phases où le poisson est zooplanctonophage, ainsi que durant la phase finale où il est benthophage; en revanche, la capacité de retenir les proies limite le taux d'ingestion maximal durant la phase avancée de la période de zooplanctonophagie. Nos résultats laissent aussi croire à l'existence de corrélations tant positives que négatives dans la morphologie entre les niches alimentaires et ils indiquent qu'il se produit des compromis morphologiques à l'intérieur des niches ontogénétiques.

[Traduit par la Rédaction]

Introduction

In size-structured populations, growing consumers are generally expected to change their ecological niche with respect to prey type, prey size, or habitat type to meet their increasing basic metabolic demands and to optimise their ecological foraging performance over ontogeny (e.g., Werner and Gilliam 1984; Persson 1988; Werner 1988). This ontogenetic increase in size imposes a number of scaling constraints, including physical, mechanical, and ecological, on organisms. These combined determine the animal's ability to consume resources, and together with environmental factors, they control the organism's size-specific competitive ability (Persson et al. 1998).

One of the factors affecting foraging rate is the animal's morphology, and it has been argued that the foraging mode is one of the parameters that will have the largest effect on fitness (Schluter 1995). Thus, animals that shift to other resources during ontogeny show concurrent changes in morphology and behaviour in order to optimise their competitive

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J. Hjelm.[1] National Board of Fisheries, Institute of Marine Research, Turistgatan 4, P.O. Box 4, Lysekil, Sweden, and Animal Ecology, Department of Ecology and Environmental Science, Umeå University, SE-901 87, Umeå, Sweden.
G.H. van de Weerd and F.A. Sibbing. Experimental Zoology Group, Wageningen Institute of Animal Sciences, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, the Netherlands.

[1]Corresponding author (e-mail: joakim.hjelm@fiskeriverket.se).

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ability in each specific niche (Wainwright 1991; Robinson and Wilson 1996; Persson et al. 1998). Organisms that switch resources or habitats are often subject to potentially conflicting selective pressures, which may result in reduced performance in contrast to organisms that specialise in only one niche (Werner and Gilliam 1984; Persson 1988; Werner 1988). This scenario, called the paradox of generalists or ontogenetic trade-offs in morphology, implies that generalists are less capable of handling a particular prey compared with specialists (Robinson and Wilson 1996; Sibbing and Nagelkerke 2001). Despite the frequent occurrence of ontogenetic niche shifts in consumers, the costs of these shifts in constraining morphological adaptations are poorly understood.

The relationship between morphological variation and feeding performance between and within species has been the focus of many studies (West-Eberhard 1989; Skúlason and Smith 1995; Sibbing and Nagelkerke 2001). However, few studies have linked feeding performance and changes in body morphology over ontogeny (but see Mittelbach et al. 1999; Hjelm et al. 2000, 2001). As outlined above, there may exist morphological trade-offs (i.e., negative correlation between stages) in organisms between niches (Schluter 1995; Robinson and Wilson 1996; Hjelm et al. 2001). However, it is also possible that there are traits, or combinations of traits, that show a positive correlation over ontogeny. For example, small fish may be limited early in life by oral gape size, but as they grow in size, they may escape this morphological limitation. With a further increase in size, they may include new types of larger prey to maintain positive growth and eventually become oral gape limited again. The size of prey relative to the size of the predator will in this case be the structural factor limiting prey intake instead of prey type per se (Persson 1988; Wainwright and Richard 1995; Mittelbach and Persson 1998).

In this study, we focus on the relationship between morphology and performance during growth of roach (*Rutilus rutilus* L.), a common Eurasian fish. The main objective was to explore the functional link between two performance aspects, foraging efficiency and ontogenetic diet shift, and the morphological development over ontogeny. Morphological measurements included structures of the feeding apparatus as suggested by the food-fish model (FFM) of Sibbing and Nagelkerke (2001) and body shape (landmark analysis). The foraging efficiency was derived from Hjelm and Persson (2001), whereas data on the ontogenetic diet shift were collected during a 2-year field study of a natural roach population.

Methods

System studied

The study was performed in Lake Kassjö situated ~25 km northwest of Umeå, central Sweden. The lake has an area of 10 ha with a maximum depth of 12 m and is relatively productive for this part of Sweden (Table 1). The littoral zone is covered with different species of macrophytes, such as *Calla palustris*, *Phragmites australis*, *Equisetum* sp., and different species of *Carex*. The dominant submerged plants are *Nuphar lutea*, *Nymphaea alba*, and *Potamogeton perfoliatus*. The submerged macrophytes cover almost the entire shoreline to a depth of 2–2.5 m. Fish species in the lake are roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), pike (*Esox lucius*), ruffe (*Gymnocephalus cernuus*), and smelt (*Osmerus eperlanus*). The lake has only one outlet and no inlet.

Table 1. Lake characteristics: chlorophyll *a* content (used as a measure of productivity) and the biomass of predator sensitive macroinvertebrates (PSM) in Lake Kassjö during the survey.

	Chlorophyll *a* ($\mu g \cdot L^{-1}$)		PSM ($mg \cdot m^{-2}$)	
	July	September	Littoral	Pelagic
1997	8.1	6.4	236 (8–36)	22 (7–13)
1998	8.4	6.8	208 (7–22)	19 (8–15)

Note: Numbers within brackets show the size (length in mm) range of macroinvertebrates.

Fish sampling

The fish fauna was sampled in both 1997 and 1998 on two dates (June and September) to estimate the biomass and size–age distribution. The gill nets (standard survey-link type) were placed at three different depths (1.5, 4, and 8 m, three nets at each depth). Each gill net was 39 m long × 1.5 m high and consisted of 14 sections of 3 m with mesh sizes 75, 60, 50, 43, 38, 33, 30, 25, 22, 16.5, 12.5, 10, 8, and 6.25 mm (knot to knot). The nets were set for 24 h. We expressed the biomass of fish as catch per net over a 24-h period (catch-per-unit-effort, CPUE). Captured fish were measured to the nearest millimetre total length (TL) and weighed to the nearest 0.1 g. All fish were deep-frozen for later analysis.

Roach diet

Gut (intestine) contents were analysed using a dissection microscope. In a subsample of roach ($N = 43$ and 51 in 1997 and 1998, respectively, including both June and September samplings), all invertebrates were classified to the lowest taxonomic level possible, counted, and measured to the nearest 0.01 mm. The lengths of all benthic macroinvertebrates prey were converted to dry mass by using length – dry mass regressions (see below). After removal of animal material in the gut, the weight of plant material and detritus was determined. Plant material and detritus was dried at 60°C for 12 h and weighed to the nearest 0.001 g and their proportion was determined.

To determine the relative prey size with respect to predator gape as a function of roach size, captured roach were divided into 20-mm size classes, ranging from 40 to 220 mm. For roach smaller than 150 mm (diet shift observed), zooplankton was used to determine average prey size, even though some benthic invertebrates were present in the diet, because zooplankton was dominant and represents the smallest, most difficult prey to handle. For roach larger than 150 mm, only benthic macroinvertebrates were used to determine average prey size, because for them benthic prey was dominant and represents the largest, most difficult prey to handle. A mirror diet analysis with all of the groups present in the diet showed a similar pattern but is not included. Length of benthic macroinvertebrates and zooplankton was recalculated to prey width, as prey width is more important than length for determining gape limitation. The prey width of benthic macro-

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invertebrates was calculated from data on chironomids and *Sialis* spp. length–width relationships (S. Diehl, Department of Biology II, Aquatic Ecology, Ludwig-Maximilians University Munich, Germany, unpublished data). This gives a coarse but fair estimate of prey width, because by combining the length–width relationships in these two species, the relationship is continuous over the length range of prey items in the diet.

Selectivity for benthic macroinvertebrates and zooplankton

To have a qualitative measure of the benthic fauna, benthic macroinvertebrates were sampled once in June in both years, using an Ekman dredge (total area 0.063 m^2). The benthic macroinvertebrates were sampled in the open-water (water depth 6–8 m, $N = 6$) and littoral (water depth 1.5–2 m, $N = 6$) zones. The samples were sieved through a 0.5 mm mesh net, preserved in 70% ethanol, and stained red with Rose Bengal. In the laboratory, invertebrates were classified to taxon and counted. The length of all individuals was measured using an image analyser system (Optilab, Graphtech, France). Lengths (area for chironomids) were converted to biomass by using length (area) – dry mass regressions (Persson and Greenberg 1990; Persson et al. 1996; J. Andersson, Department of Ecology and Environmental Sciences, Umeå University, Umeå, Sweden, unpublished data). Benthic macroinvertebrates were separated into two groups. One group consisted of macroinvertebrates living on the substrate, which includes organisms such as *Ephemeroptera*, *Trichoptera*, *Odonata*, and *Asellus* sp. All of these taxa are relatively large, conspicuously visible, and thus sensitive to fish predation. The other group consisted mainly of chironomids and oligochaetes, which are invertebrates living in the sediment substrate, rendering them less sensitive to visual fish predation (for a discussion of sensitivity to fish predation, see Persson et al. 1996). The selectivity for predator-sensitive benthic macroinvertebrates was calculated using Ivlev's electivity index:

$$(1) \qquad E_i = \frac{r_i - n_i}{r_i + n_i}$$

where r_i is the percentage of prey type i in the diet and n_i is percentage of the same prey type i in the environment (Ivlev 1961). The index ranges from −1 to +1, with positive values indicating preference and negative values indicating avoidance. The calculated index was an average of the diet and benthic samplings over the two seasons.

To have a qualitative measure of the zooplankton fauna, the lake was sampled four times (June, July, August, and September) in the pelagic and the littoral zone each year. For the littoral zone sampling, a hand net (60-µm mesh, 240 mm in diameter) was towed 3 m just below the surface (total volume filtered 138 dm^3) at a speed of 0.5 $m \cdot s^{-1}$ ($N = 3$). For the vertical pelagic sampling, a tow net (60-µm mesh, 150 mm in diameter) was drawn from 1 m above the lake bottom to the surface (9 m, $N = 3$). Zooplankton samples were preserved in Lugol's solution. In the laboratory, zooplankton were classified to taxon and the length of 10 individuals of each taxon in each sample was measured using an inverted microscope (Leica MZ8). This average length was used to transform the density of zooplankton to biomass using length–biomass regressions (see Persson et al. 1996). Roach size-dependent preference for large zooplankton (>0.60 mm, mainly *Daphnia*) was calculated with Ivlev's electivity index (eq. 1). The calculated index on large zooplankton was an average of the diet and zooplankton samplings over the two seasons. The zooplankton size used (>0.60 mm) was determined from measurements on the spacing between gill rakers, which suggested that larger roach should be able to retain these zooplankton.

Morphometrics

To describe important species-specific changes in morphology over ontogeny, we measured both different structures in the head and the overall body shape.

Feeding apparatus

To study the diversity of the feeding apparatus and its ecological significance in Cyprinidae, Sibbing and Nagelkerke (2001) selected a set of structures with established functional significance in foraging and processing of different food types (full description of the FFM in Sibbing and Nagelkerke 2001). The FFM explains the linkage between functional morphology of the fish feeding apparatus and food characteristics (size, location, velocity, mechanical, and chemical). The following structures were measured as explained in Sibbing and Nagelkerke (2001) and further below: (1) weight of dried pharyngeal jaw weight, average between left and right; (2) pharyngo-opercular volume; (3) oro-buccal volume; (4) length of the ligament 2 between lower and upper jaw (maxilla; Ballintijn 1972); (5) oral gape size (maximum diameter read from a measuring cone inserted into the fully protruded mouth; Hjelm et al. 2000); (6) pharyngeal jaw symphysis length; (7) length of medial process on the premaxilla; (8) length of hyoid bar (fused ceratohyal and hypohyal); (9) rostro-caudal maxilla length; (10) inter-raker distance of first left gill arch; (11) head length; (12) lower jaw length; (13) protrusion length, extension of upper lip at full opening oral gape; (14) gill-raker length of the second left gill arch; (15) palatal organ length (measured on the castings) between its anterior rim and chewing pad; and (16) eye diameter. These structures were chosen to represent all prey capture and food-handling processes. Structures in roach (>60 mm TL) were measured with either a digital or an analogue calliper. Structures in specimens smaller then 60 mm were measured using a stereomicroscope (Leica MZ8) with ocular micrometer.

Oro-buccal and pharyngo-opercular volumes were obtained by making castings of the oro-buccal and the pharyngo-opercular cavities. These castings were made by placing the fish in a vertical position (head upward) with the mouth fully open and protruded. The branchial arches with gills were largely removed by cutting them above the epibranchials and below the ceratobranchials. Immersing the removed arches in a water-filled cylinder yielded the volume to be subtracted from the pharyngo-opercular volume. Maximum expansion of the mouth cavity was obtained by pulling the hyoid in a ventral direction according to van de Weerd (2000). The opercular bones were expanded to a position in which the opercular flaps made contact with the pectoral girdle. All cavities were filled with dental paste (Xantopren L, Heraeus). The casting was removed by dissecting the head

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Fig. 1. Landmarks used in the analysis of roach (*Rutilus rutilus*) body shape. The mouth was fully protruded during measurements.

and then was cleaned and dried. Weighing the casts and using the density of the dental paste gave the required volumes. The pharyngo-opercular volume was measured by cutting away the oro-buccal part of the cast at the point where the hyoid connects to the hyomandibula (a clear imprint on the cast). The imprint on the cast was used to measure the length of the palatal organ, between its rostral rim and the chewing pad.

All inter-raker distances were measured on the medial side of the first left gill arch. Gill raker lengths were measured on the opposite lateral side of the second left gill arch. Both measurements were made using a stereomicroscope (Leica MZ8) with an ocular micrometer as described by van den Berg et al. (1992).

The morphometric data on the 16 head-related structures were analysed using principal component analysis (PCA, SPSS 7.5, no rotation on log data, covariation matrix). A total of 86 fish were used in the analysis. The values of the morphological parameters were log-transformed, and the weights and volumes were linearised before analysis by regressing them to $L^{1/3}$. The three structures with the highest loadings (positive or negative) were used in the results. A mirror analysis of the 16 morphological structures was performed on the correlation matrix, but resulted in a similar pattern and is not shown here.

Body morphology

In total, 22 landmarks (Fig. 1) were used to describe the body morphology (shape) of the fish. The landmarks were measured by viewing roach ($N = 317$) through a microscope (Leica MZ8) and projecting the image onto a digitising tablet (Summasketch III). Each fish was positioned with a fully protruded upper jaw and pinned with needles laterally with the fins spread. The measured fish ranged from 0+ to 4+. The landmarks on the fish were collected using DS-Digit (Slice 1994), and the measurements were repeated three times per fish to determine the measurement error. The measurement error for each landmark was in all cases less than 5%. The average value per individual (three measurements) was translated, scaled, and rotated by generalized least square procustes fit using the GLS option in the GRF-ND program (Slice 1993). This enabled us to retain the centroid size as an independent variable and as a measure of size variation. The nonuniform components were then analysed with relative warp analysis using tpsRelw (Rohlf 1993). This procedure removes all isometric shape changes and leaves allometric shape variation in morphology, which later can be analysed. Then tpsRelw was used to calculate individual scores for each individual and for each relative warp. Relative warp can be compared with principal components in traditional statistical analysis and is used to explain the variation in the individual morphology compared with the consensus specimen. From this analysis, four body shape components (RW1–RW4) were derived and used for sequential statistical analysis (analysis of covariance (ANCOVA) with size as covariate; Table 2).

Table 2. The relationship between size (total length) and the different body morphological components (relative warp units, RW1–RW4).

Relative warp (RW)	r^2	SS	df	F	P
RW1	0.11	3.195×10^{-6}	1,316	1.97	<0.001
RW2	0.43	1.436×10^{-4}	1,316	249.2	<0.001
RW3	0.001	3.265×10^{-8}	1,316	0.105	0.75
RW4	0.001	4.935×10^{-8}	1,316	0.196	0.66

Note: Note that RW1 and RW2 are related to size, whereas RW3 and RW4 are not.

Results

Resources

Benthic macroinvertebrates

The biomass of predator-sensitive macroinvertebrates (PSM) did not differ between years, but the density was higher in the littoral zone than in the pelagic zone (two-way analysis of variance (ANOVA): habitat, $F_{[1,20]} = 31.6$, $P < 0.001$; year, $F_{[1,20]} = 0.36$, $P = 0.56$; year × habitat, $F_{[1,20]} = 0.25$, $P = 0.63$; Table 1).

Zooplankton

As generally found in systems like these, the total biomass of zooplankton was relatively high in June, decreased in July and August, and increased again in September (Fig. 2*a*). Similarly, the average size of zooplankton was relatively large in June, decreased in July and August, and increased again in September (Fig. 2*b*). The total biomass of zooplankton did not differ between years (two-tailed paired t test: $t = 1.23$, $P = 0.31$). The biomass of zooplankton was higher in the pelagic zone than in the littoral zone (repeated-measures ANOVA: habitat, $F_{[1,2]} = 32.5$, $P = 0.029$), and the initial decrease was higher in the pelagic habitat than in the littoral habitat (repeated-measures ANOVA: habitat × time, $F_{[3,6]} = 7.1$, $P = 0.021$). The mean zooplankton size was somewhat larger in the pelagic zone than in the littoral zone, but the decrease in zooplankton size was not related to habitat (repeated-measures ANOVA: habitat, $F_{[1,2]} = 205$, $P = 0.005$; habitat × time, $F_{[3,6]} = 0.86$, $P = 0.51$). *Daphnia* was present during the whole season and dominated the zooplankton biomass in Lake Kassjö in September 1998.

Roach biomass, diet, and foraging efficiency

Biomass

Roach dominated the fish biomass in both the littoral and the pelagic habitats in both years (relative biomass of roach: 1997, 69%; 1998, 62%). Perch and pike biomasses averaged 28 and 3%, respectively, and the biomass of ruffe and smelt constituted less than 1% each year. Roach biomass was four

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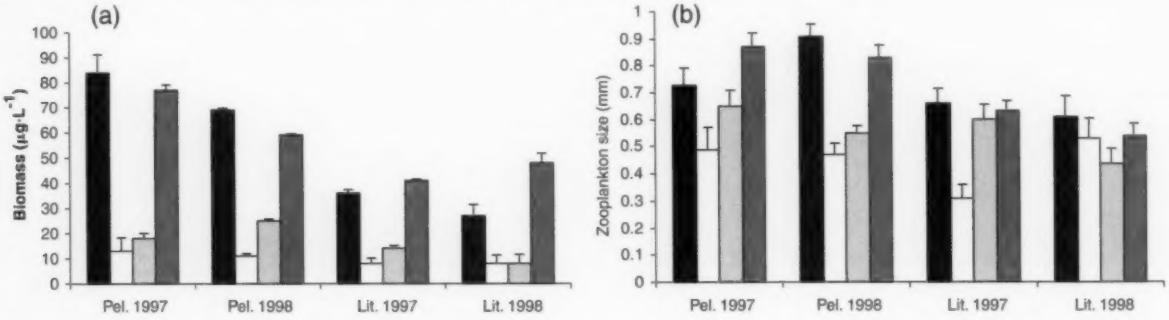
Fig. 2. (*a*) The total biomass of zooplankton (mean + 1 standard deviation, SD) in Lake Kassjö at the different sampling locations (littoral (Lit.) and pelagic (Pel.)) in 1997 and 1998. The total biomass of zooplankton did not differ between years (two-tailed paired *t* test: $t = 1.23$, $P = 0.31$). (*b*) The average size of zooplankton (mean + 1 SD) in Lake Kassjö at the four sampling locations in 1997 and 1998: June, solid bars; July, open bars; August, lightly shaded bars; September, darker shaded bars.



times higher in the littoral zone than in the pelagic zone. The mean size of roach was higher in the pelagic zone (averaging 154 mm) than in the littoral zone (averaging 112 mm) (two-tailed *t* test: $t = 2.0$, $P = 0.023$). The oldest roach found in the lake was 6 years old but the lake was dominated both years by 2-year old roach.

Diet

The diet was similar in 1997 and 1998 with small roach feeding mainly on zooplankton and larger roach feeding mainly on benthic macroinvertebrates (Fig. 3*a*). The diet shift from zooplankton to benthic macroinvertebrates was quite discrete and occurred in both years around a roach size of 140–160 mm. The amount of detritus and plant material gradually increased with roach size (Fig. 3*b*). The diet choice corresponded to a preference for PSM with increasing roach size (Fig. 4), a monotonic trend, not significantly different from a linear relationship (partial *F* test: $P > 0.05$). The electivity index for large zooplankton (mainly *Daphnia*) suggested that medium-sized roach had the strongest preference for *Daphnia*, whereas small and large roach showed avoidance for large zooplankton (nonlinear regression: $r^2 = 0.48$; partial *F* test for difference from a linear relationship: $P < 0.01$; Fig. 4). The proportion of zooplankton in the diet of roach caught in the littoral zone was lower than in pelagic roach (ANOVA on residuals from the proportion of zooplankton over size: $F_{[1,125]} = 15.87$, $P < 0.001$). Roach relative prey width (prey width – predator gape width) initially decreased with roach size up to the diet shift to macroinvertebrates and thereafter increased (Fig. 5). Prey size was almost 20% of gape size at 50 mm, around 10% for roach 110–130 mm, and approximately 20% for larger roach (>190 mm).

Foraging efficiency

The attack rate of roach on 0.5-mm zooplankton as a function of roach size was recalculated from Hjelm and Persson (2001). The capture rates for all size classes of roach and zooplankton as a function of zooplankton densities were well described by a Holling's type-2 functional response curve (Hjelm and Persson 2001). The attack rate of roach as a function of size showed a hump-shaped relationship with a maximum attack rates around 150 mm independent of zooplankton size (Fig. 6). The maximum attack rate occurred at the same size interval as roach shifted diet to macroinvertebrates in Lake Kassjö (compare Figs. 3*a* and 6). Fur-

Fig. 3. (*a*) The biomass of zooplankton (%) and benthic macroinvertebrates (%) in the diet of roaches (*Rutilus rutilus*) after removal of all biomass of plant material and detritus. (*b*) The biomass (%) of total diet that was detritus and plant material (total %). Solid triangles represent zooplankton, open circles represent benthic invertebrates, and open squares represent plant materials and detritus.

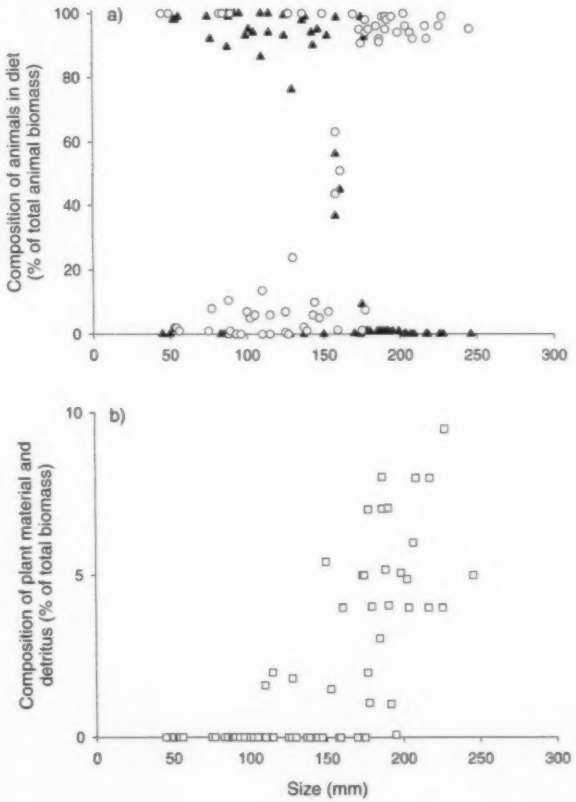


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Fig. 4. Roach (*Rutilus rutilus*) selectivity index (Ivlev's; proportion in the diet compared with what was found in the environment) for large zooplankton (*Daphnia* > 0.6 mm) and predator-sensitive macroinvertebrates as a function of body size. The solid line shows the relationship between roach body size and selectivity on predator-sensitive macroinvertebrates (r^2 = 0.47), whereas the broken line shows the relationship between roach body size and selectivity on large zooplankton (r^2 = 0.60). Solid triangles represent zooplankton, and open circles represent predator-sensitive invertebrates. The dotted line denotes the diet shift from zooplankton to benthic macroinvertebrates.

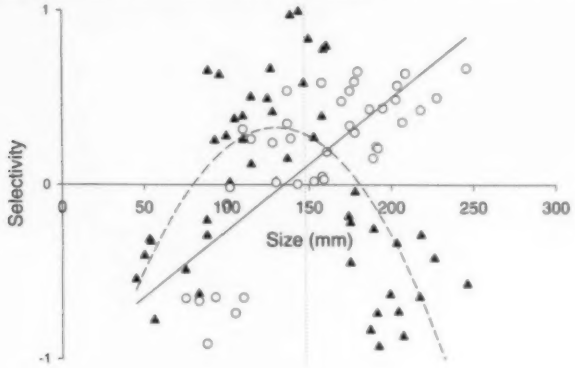
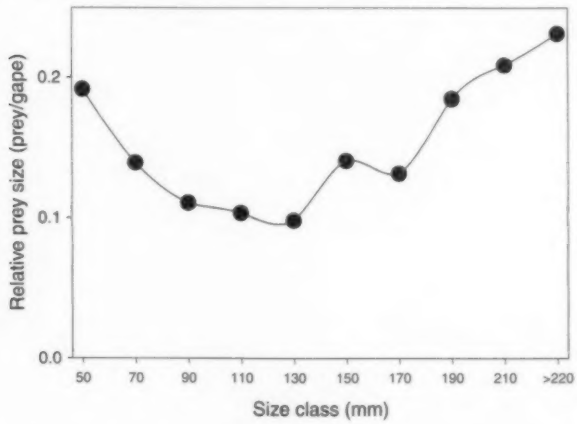


Fig. 5. The ratio of prey width and predator gape. Note that over the zooplanktivorous feeding niche, only zooplankton has been used in the ratio, whereas in the benthivorous feeding niche, only benthic macroinvertebrates have been used.



thermore, the attack rate on *Daphnia* was positively related to the observed selectivity on *Daphnia* by roach in Lake Kassjö (correlation coefficient r = 0.87).

Feeding apparatus and body shape

Feeding apparatus

By using a PCA, the original 16 structures were reduced to three principal components (PC), which together explained 98% of the total head variation (PC1 = 59%, PC2 = 36%, and PC3 = 3%). PC1 was positively correlated to size (regression: r^2 = 0.94, F = 1105, P < 0.001), whereas PC2 was

Fig. 6. The principal component analysis (PCA) for the different morphological structures in the feeding apparatus for differently sized roach (*Rutilus rutilus*). PC1 (59%; open squares) and PC2 (36%; solid circles) are shown where PC1 and PC2 have a slope significantly different from zero. The solid line shows the size-dependent attack rate for roach on a 0.5-mm zooplankton derived from Hjelm and Persson (2001).

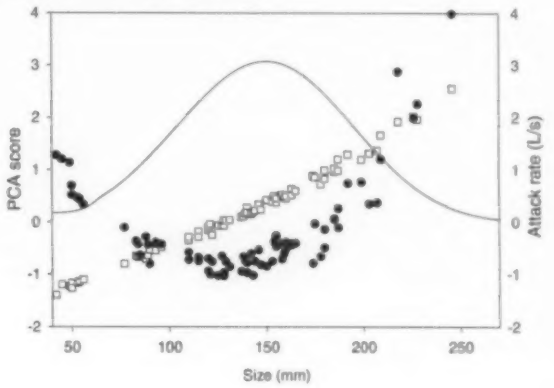


Table 3. Loadings of morphological structures included in the principal component analysis for the two first principal components, together explaining 95% of the variation.

	PC1	PC2
Pharyngeal jaw weight	0.831	0.510
Pharyngo-opercular volume	0.901	0.442
Oro-buccal volume	0.918	0.388
Ligament 2 length	0.982	0.015
Gape size	0.996	–0.010
Protrusion length	0.982	–0.017
Symphysis length	0.992	–0.026
Process premaxilla length	0.987	–0.064
Hyoid length	0.988	–0.060
Maxilla length	0.991	–0.064
Inter-raker distance	0.980	–0.083
Head length	0.993	–0.087
Lower jaw length	0.985	–0.106
Eye diameter	0.923	–0.107
Gill raker length	0.979	–0.151
Palatal organ length	0.957	–0.281

Note: Underlined values show high and low loadings, respectively, for PC1 and PC2.

U-shaped with the highest scores for small roach (<50 mm) and large roach (>200 mm) (Fig. 6). The relationship between the morphometric scores of PC2 and roach size was significantly different from a linear relationship (partial F test: P < 0.01). Structures that had highest positive loadings in PC1 were oral gape size, head length, and symphysis length of the pharyngeal jaw (Table 3). Structures that had highest positive loadings in PC2 were pharyngeal jaw weight, pharyngo-operculum volume, and oro-buccal volume. Structures that had highest negative loadings in PC2 included palatal organ length, gill-raker length, and eye diameter. In the

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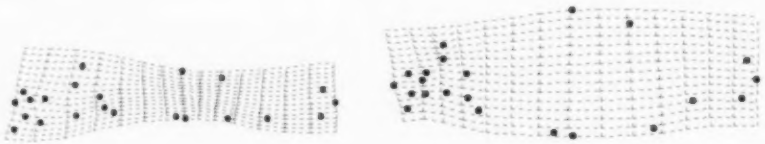
Fig. 7. The overall body shape for small roach (*Rutilus rutilus*; 45 mm) and large roach (220 mm). Note that the mouth is fully protruded. Landmarks are shown in Fig. 1.



Roach 45 mm Roach 220 mm

Table 4. (*a*) The overall MANCOVA (between factors) of the four body morphological components derived from the relative warp analysis (RW1–RW4; Wilk's λ), with habitat (pelagic and littoral habitat) as factor and size (total length) as a covariate. (*b*) The within-factors analysis (MANCOVA) on the morphological components (RW1–RW4).

(*a*) The overall (between factors) MANCOVA.

	F	df	*P*
Size	58.561	4,311	<0.001
Habitat	45.391	4,311	<0.001

(*b*) The within-factors MANCOVA.

		Type III SS	MS	df	*F*	*P*
Size	RW1	3.17×10^{-5}	3.17×10^{-5}	1,314	26.4	<0.0001
	RW2	7.22×10^{-5}	7.22×10^{-5}	1,314	191.1	<0.0001
	RW3	5.95×10^{-8}	5.95×10^{-8}	1,314	0.19	0.66
	RW4	2.12×10^{-7}	2.12×10^{-7}	1,314	0.85	0.36
Habitat	RW1	4.36×10^{-6}	4.36×10^{-6}	1,314	3.63	0.05
	RW2	6.28×10^{-5}	6.28×10^{-5}	1,314	166.3	<0.0001
	RW3	4.61×10^{-8}	4.61×10^{-8}	1,314	0.15	0.70
	RW4	5.32×10^{-7}	5.32×10^{-7}	1,314	2.12	0.14

different regressions between principal components and size, the standard residuals were used to investigate whether roach caught in the two different habitats differed with respect to morphology. However, there was no difference in individual residuals between roach caught in the pelagic habitat and those caught in the littoral habitat (one-way ANOVA: $P > 0.4$, 0.5, and 0.8 for PC1, PC2, and PC3, respectively).

Body

Roach exhibited allometric growth and the main change in body shape over ontogeny was that smaller individuals had a terminal position of the oral gape, a relatively larger head, a slender body, and a narrower caudal peduncle (Table 2). Larger roach had a subterminal position of the oral gape, a relatively smaller head, and high body shape, as well as a slightly higher caudal peduncle (Fig. 7). The relative warps (RW1–RW4) used explained 77.4% (RW1 = 36.7%, RW2 = 27.7%, RW3 = 7.8%, and RW4 = 5.2%) of the total body shape variation. There was no overall difference in body shape between years (multiple analysis of covariance (MANCOVA), size as a covariate: $P > 0.5$). RW1 explained a difference in body torque in which small roach had a U-shaped body and large roach had the inverse shape. RW2 mainly explained the variation in body height and head size. RW3 suggested that small roach were more slender than large roach and that small roach had a thinner and longer head than large roach. RW1 and RW2 were negatively related (significantly) to roach size, whereas RW3 and RW4 were unrelated to size, which indicates that the body shape described by RW1 and RW2 are largely determined by intrinsic growth, whereas body shape described by RW3 and RW4 are set by extrinsic environmental factors (Table 4). Irrespective of size, roach caught in the littoral zone had a more subterminal oral gape position and a higher body shape compared with roach caught in the pelagic zone (Table 4*a*). However, the difference between habitats was only present for RW1 and RW2 (Table 4*b*). There was no correlation between body morphology (described by relative warps) and head morphology (described by principal components) ($r^2 < 0.1$ in each case).

Discussion

Ontogenetic diet shift, foraging performance, and morphological changes

The diet of small roach (<150 mm TL) in Lake Kassjö mainly consisted of zooplankton, whereas roach larger than 150 mm mainly fed on benthic macroinvertebrates. This size-related diet shift corroborates results from other studies (Persson 1983; Johansson and Persson 1986; Horppila et al. 2000), even though the actual size at diet shift differs among lakes. Also in a pond experiment, Hjelm and Persson (2001) found that 150-mm roach feed more on zooplankton compared with 230-mm roach. They also showed that the relation-

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ship between foraging performance (attack rate) on 0.5-mm zooplankton and roach body size was hump-shaped with a maximum around 150 mm (TL). The hump-shaped size-dependent foraging performance on zooplankton together with an increased foraging performance on benthic prey at large size provide a mechanistic explanation for why growing roach change their ecological niche with respect to prey type and how they optimise performance to maximise energy gain (Werner and Gilliam 1984; Persson 1988; Werner 1988). We suggest two factors explaining diet shifts in roach. First, larger roach (>200 mm), because of their size, may not be able to meet their increasing basic metabolic needs by feeding on small zooplankton at the resource levels observed in Lake Kassjö. Hence, they need additional resources (Hjelm and Persson 2001). This view is supported by medium-sized roach feeding more selectively on larger zooplankton (*Daphnia*) than small roach, whereas large roach foraged more selectively on macroinvertebrates. Secondly, the diet shift of roach is facilitated by the following allometric morphological changes in both body shape and feeding apparatus.

During growth, roach increased body depth with size, rendering it more suited for benthivorous feeding (Webb 1988; Robinson et al. 1993; Hjelm et al. 2000). Feeding on benthic macroinvertebrates demands high manoeuvrability rather than a fast attack. Manoeuvring at foraging requires a high and laterally compressed body, a subterminal mouth, and large mobile pectoral fins. In contrast, fusiform bodies with a terminal mouth optimise zooplanktivory (Webb 1975; Norton 1995; Robinson and Wilson 1996). Additional support for this assumption comes from evidence that roach caught in the pelagic zone had a more fusiform body shape than littoral roach. Accordingly, pelagic roach had a higher proportion of zooplankton in their diet compared with roach from the structurally complex littoral zone. This, in turn, implies not only that roach show a general increase in body depth over ontogeny, but that this is reinforced by a specific habitat and diet use, similar to other species of fish (see Robinson et al. 1993; Hjelm et al. 2001; Sibbing and Nagelkerke 2001).

PCA of changes in the feeding apparatus showed that the first principal component (PC1) was positively correlated to size, which implies that the different feeding structures mainly increased in size. Structures with highest loadings in PC1 included head length, oral gape size, and symphysis length of the pharyngeal jaws, which together are optimised for intake and mechanical breakdown of large, stiff prey (Sibbing and Nagelkerke 2001) during growth. In contrast, the relationship between PC2 and body size was U-shaped with a minimum around 140 mm. Structures with high positive loading in PC2 included pharyngeal jaw weight and oro-buccal and pharyngo-opercular volumes. Structures with high negative loadings were eye diameter, gill-raker length, and palatal organ length. These structures with high loadings are involved in different feeding actions including prey detection, intake, size and taste selection, and prey mastication (Sibbing and Nagelkerke 2001). The U-shaped relationship between PC2 and size was the inverse of the size-dependent attack rate. PC2 suggests that both intake limitations (buccal and pharyngo-opercular volumes) as well as food processing capacity (pharyngeal jaw weight) are important during early ontogeny characterised by low foraging performance on zooplankton. In contrast, prey retention ability (gill-raker length and palatal organ length) may be limiting at maximum attack rates (Persson 1987; Noakes and Godin 1988). Experiments have shown that gill rakers play a prominent role in retention of small particles and that the palatal organ length is important for internal taste and size selection (Sibbing et al. 1986; Hoogenboezem et al. 1990; Osse et al. 1997). Sibbing et al. (1986) and Osse et al. (1997) have shown that the surface area of the palatal organ and its taste-bud density play an important role for separating prey and nonprey particles. Thus, the palatal organ may be important for collecting and transporting relatively small zooplankton from the branchial sieve in medium-sized roach. Hjelm and Persson (2001) showed that roach maximum attack rate on different zooplankton sizes is irrespective of its size, which indicates that the maximum foraging rate is determined by retention ability rather than by the predator body size or branchial sieve (Hoogenboezem et al. 1990; van den Berg et al. 1992).

Ontogenetic trade-offs between or within niches

It has become clear that predator size limits size and type of prey, e.g., zooplankton, and may favour the utilisation of others, e.g., macroinvertebrates. The animal's morphology is subjected to trade-offs across different niches (Werner 1988). We showed that the feeding apparatus of roach changes related to a diet shift. Roach is subjected to contradictory selective pressures during ontogeny (Werner and Gilliam 1984; Robinson et al. 1996; Sibbing and Nagelkerke 2001).

In contrast to the commonly viewed trade-off in body morphology between different niches, our study points to both a possible morphological trade-off within each of the two feeding niches, as well as positive correlations between niches. As suggested, the structures important for both zooplankton feeding during early ontogeny (roach size < 50 mm) and benthic macroinvertebrate feeding later in ontogeny (roach size > 200 mm) both showed high scores in PC2. Thus, there exists a positive correlation in morphology between these two niches. Based on functional assumptions from previous work on cyprinids (Sibbing and Nagelkerke 2001), the morphological structures with high loadings in PC2 can be hypothesised to aid both small and large roach handle and process relatively large prey (prey width – predator size ratio). The diet analysis revealed that small and large roach feed on comparable prey sizes (relative to gape size), even though their absolute size differed considerably. It can be hypothesised that the morphology in small and large roach may be optimised similarly but for different prey types and that prey size is more important than prey type per se (Wainwright and Richard 1995; Mittelbach and Persson 1998; Sibbing and Nagelkerke 2001). In contrast, medium-sized roach fed on small prey relative to their own size. Handling relatively small prey items requires a different set of structures involving lower demands for mechanical breakdown but, at the same time, a higher retention capacity by optimising gill rakers and palatal organ (Hoogenboezem et al. 1990; van den Berg et al. 1992; Osse et al. 1997). The observed selectivity pattern for zooplankton agrees with this interpretation, as medium-sized roach (that were retention limited) showed a strong positive selection for large zooplankton,

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whereas smaller roach (that may be intake limited) showed avoidance of large zooplankton.

The feeding apparatus in fish is a complex structure composed of integrated functional components serving different actions during prey capture and food processing. Internal feeding structures share the same physical space but need to be optimised to handle different prey types as a function of fish size. It is likely that the feeding apparatus is subjected to different physical trade-offs over ontogeny and even within a single niche because of changes in relative prey size. Small and large roach that feed on relatively large prey need to create large suction, in contrast with medium-sized roach feeding on relatively small prey, optimising retention and processing structures. For example, the size of the palatal organ competes for overall volume in the mouth cavity, and consequently, if volume is needed for suction, taste selection will be adversely affected (Osse et al. 1997). Another example would be small and large roach that feed on relatively large prey and need to create a large suction force by fast and voluminous expansion of the mouth cavity. However, long gill rakers and narrow inter-raker distances, which are used to retain relatively small prey (both shown in PC2 and PC3), will reduce suction ability by increasing flow resistance over the branchial sieve. Thus, a hydrodynamic trade-off is present between sieving and suction (van den Berg et al. 1992). The morphological changes over ontogeny seen mainly in PC2 suggest that roach may be subjected to a morphological trade-off within a single resource niche (zooplankton) as a function of relative prey size. PC2 also contrasts with the general view that zooplankton and benthic feeding have different size-dependent structural demands and that morphology should be subjected to trade-offs between these niches (Schluter 1995; Robinson and Wilson 1996; Hjelm et al. 2000).

To conclude, we suggest that roach attack rate, which is the integrated result of different actions during the predation cycle, and diet niche shifts are closely related to overall body shape and the morphology of the feeding apparatus. Furthermore, our study indicates that morphological trade-offs may exist between niches as well within a single niche. Even though there is a general view that morphology and performance is strongly linked, there are very few studies that have considered all of these factors. We also suggest that our findings and discussion may be general for other fish taxa, which points to the need to consider changes over ontogeny when linking performance and functional morphology.

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Implications of life-history invariants for biological reference points used in fishery management

Erik H. Williams and Kyle W. Shertzer

Abstract: Fish harvest policies typically rely on biological reference points for measures of a stock's status. We examine three common biological reference points based on fishing mortality rates corresponding to maximum sustainable yield with an age-structured deterministic model. We incorporate invariant life-history relationships into the model to maintain parsimony and focus model parameters on biologically plausible parameter space. A wide range of biological and fishery characteristics were used in the model so that our results pertain to the management of virtually any exploited population. Results indicate that two biological reference points based on spawning biomass are insensitive to life-history parameters, whereas one based on natural mortality is highly sensitive. All three depend largely on the choice of a stock–recruitment function and on steepness, a measure of the population growth rate. For each of the three, values have been previously proposed that were intended to safely apply to all fisheries; our results show that no such universal values exist. We recommend determining stock–recruitment functions a priori, establishing biological reference points on steepness explicitly and eliminating harvest policies based on the natural mortality rate altogether.

Résumé : Les politiques de récolte de poissons se basent généralement sur des points de référence biologiques comme mesures du statut d'un stock. Nous examinons ici trois points de référence biologiques d'usage courant, qui sont basés sur des taux de mortalité dus à la pêche correspondant au rendement équilibré durable, à l'aide d'un modèle déterministe structuré d'après l'âge. Nous incorporons au modèle des relations invariables du cycle biologique pour maintenir la parcimonie et nous maintenons les paramètres du modèle dans un espace de vraisemblance biologique. Une gamme étendue de caractéristiques biologiques et halieutiques est utilisé dans le modèle, si bien que nos résultats s'appliquent à presque toute population exploitée. Nos résultats indiquent que les deux points de référence biologiques basés sur la biomasse des géniteurs sont insensibles aux paramètres du cycle biologique, alors que l'autre basé sur la mortalité est très sensible. Tous les trois dépendent fortement du choix de la fonction stock–recrutement et de l'inclinaison, une mesure du taux de croissance de la population. Pour chacun des trois, on a proposé dans le passé des valeurs qui permettaient de les appliquer sans problème à toutes les pêches; nos résultats démontrent que de telles valeurs universelles n'existent pas. Notre recommandation est d'établir a priori les fonctions stock–recrutement, de baser les points de référence biologiques explicitement sur l'inclinaison et d'éliminer complètement les politiques de récolte basées sur la mortalité naturelle.

[Traduit par la Rédaction]

Introduction

Biological reference points (BRPs) are used in marine fishery management to set harvest policies. They serve as either a target of management (target reference point) or a limit of exploitation (limit reference point). Distinction between target and limit allows precautionary management in the sense of maximizing harvest while protecting the spawning potential of the stock (Prager et al. 2003).

In the role of target, BRPs are a proxy for optimal harvest when data restrictions preclude direct estimation. In the role of limit, BRPs are a harvest threshold not to be exceeded. For either role, numerous BRPs have been proposed, typically based on fishing mortality rate (F) or some measure of abundance such as spawning stock biomass (SSB) or spawning stock biomass per recruit (SSBR) (Quinn and Deriso 1999).

One common BRP relates the target or limit fishing mortality rate (F') to the natural mortality rate (M) by some constant c_1 such that $F' = c_1M$. The rationale of this "M-based" BRP is that stocks with higher M tend to have higher production rates and can therefore sustain increased fishing. It was originally proposed that $F' = M$ as a proxy for the maximum sustainable fishing rate (F_{MSY}). Current applications usually take a more risk-averse strategy by considering M an upper bound and using $c_1 < 1$ (Quinn and Deriso 1999). For example, groundfish harvest policies for the Pacific and North Pacific (PFMC) councils use $F' = 0.75M$ in cases when limited data do not allow reliable estimation of F_{MSY} (Witherell and Ianelli 1997; PFMC 2001). The rationale is of

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E.H. Williams[1] and K.W. Shertzer. Center for Coastal Fisheries and Habitat Research, National Oceanic and Atmospheric Administration, 101 Pivers Island Road, Beaufort, NC 28516, U.S.A.

[1]Corresponding author (email: Erik.Williams@noaa.gov).

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this "M-based" BRP that stocks with higher M tend to have higher production rates and can therefore sustain increased fishing. It was originally proposed that $F' = M$. Current applications usually take a more risk-averse strategy by considering M an upper bound and using $c_1 < 1$ (Quinn and Deriso 1999). For example, groundfish harvest policies for the Pacific and North Pacific (PFMC) councils use $F' = 0.75M$ in cases when limited data do not allow reliable estimation of F_{MSY} (Witherell and Ianelli 1997; PFMC 2001).

A second common BRP is based on biomass, labeled here the "B-based" BRP. The goal is to define F' such that the corresponding equilibrium biomass is a fixed fraction c_2 of the unfished biomass, $SSB_{F=F'}/SSB_{F=0} = c_2$. The ratio measures the impact of fishing on the stock's potential biomass. A larger ratio implies a stock less affected by fishing. Under simple logistic population growth (e.g., Graham-Schaefer model), the c_2 corresponding to SSB at F_{MSY} is 0.5. For the Beverton–Holt and Ricker stock–recruit functions, the c_2 corresponding to SSB at F_{MSY} is ≤0.5 (Ricker 1975). The Pacific Fishery Management Council uses a target B-based BRP of $c_2 = 0.4$ for their groundfish harvest policy (PFMC 2001).

A third common BRP is the spawning potential ratio (SPR; Gabriel et al. 1989; Goodyear 1993). SPR is the spawning stock biomass per recruit of a fished population relative to that of an unfished population. As before, the goal is to define F' such that the BRP equals some fixed fraction c_3. The SPR is then $SSBR_{F=F'}/SSBR_{F=0} = c_3$. Lower values of c_3 correspond to reduced spawning potential at higher fishing rates. Several analyses have considered potential values of c_3 for general applicability to many fisheries. Clark (1991) analyzed values of c_3 over a range of biological and fishery parameters for New England groundfish and determined $c_3 = 0.35$ would maintain sufficient spawning biomass levels and could serve as a management target. The target value was subsequently revised to $c_3 = 0.3$ by Mace and Sissenwine (1993) and $c_3 = 0.4$ based on analyses by Clark (1993) and Mace (1994). For West Coast groundfish, it appears that c_3 values as high as 0.5 are more appropriate as a management target (Clark 2002; Dorn 2002; Myers et al. 2002).

The three BRPs above, although based on different metrics (F, SSB, or SSBR), are not independent. They are related by the biology of the stock. For example, targeting F as a percentage of natural mortality has consequences for SPR in ways that depend on life-history traits. However, management typically uses a BRP without considering the implications for other potential BRPs and often unavoidably so when life-history data are limited.

In this paper, we use life-history theory to examine general applicability of the three common BRPs: M based, B based, and SPR. According to life-history theory, the evolution of demographic parameters is shaped by trade-offs (Stearns 1992). Energy allocated toward one function is energy not allocated toward another. For example, increased reproductive output may come at the cost of decreased survival. Such evolutionary constraints underlie a solid body of literature indicating invariant relationships between life-history parameters (overview in Charnov 1993). By building life-history invariants into models of stock dynamics, we are able to explore the robustness of and connections between the three harvest policies.

Our goals are to address three key questions regarding the link between life histories and BRPs corresponding to F_{MSY}. First, how are each of the BRPs constrained by invariant life-history relationships? Or conversely, once a harvest rate is specified, what does it imply about the underlying life-history parameters necessary to support it? It would be useful to know if a specific management strategy can be supported over a wide range of life histories, and if not, to which life-history parameters the strategy is most sensitive. Such information could help focus data collection. Second, how do the various harvest policies interrelate? For a given set of biological parameters, implementing a particular level of one harvest strategy automatically implies levels of the others. Commonly suggested levels may not be compatible among BRPs. Third, how sensitive are our results to deviations from the invariant life-history relationships? Although such relationships are evident across stocks, they may not describe any single stock well. A good harvest strategy should be robust to deviation from the expected life-history patterns. We explore answers to the above questions over ranges of the life-history strategy, steepness in the stock–recruit relationship, selectivity pattern, and timing of selection relative to maturity. The implications of our results apply generally to the management of virtually any stock.

Materials and methods

Model of stock dynamics

We simulate stock dynamics using a continuously structured catch-age model, similar to "dynamic pool" models standard in fishery management (e.g., Sissenwine and Shepherd 1987; Thompson 1992). A cohort of age $a = 0$ is initialized with N_0 individuals. As the cohort ages, the number of individuals in that cohort $N(a)$ decreases continuously owing to fishing (F) and natural mortality (M) rates,

$$\frac{dN(a)}{da} = -[M + Fs(a)]N(a) \quad (1)$$

where $s(a)$ is the age-dependent selectivity of fishing methods. We assume deterministic dynamics, which leads to a stable age structure in this model. Thus over the course of an individual's lifetime, integration with respect to age is equivalent to integration with respect to time. Given initial conditions $N(0) = N_0$, integration projects the number of individuals to any given age a:

$$N(a) = N_0 \exp\left(-Ma - F\int_0^a s(x)dx\right) \quad (2)$$

where x is a dummy variable of integration. Further integration across all ages provides the total yield obtained from a single cohort:

$$C = \int_a Fs(a)N(a)da \quad (3)$$

In typical catch-age models with discrete age classes and constant age-specific selectivity, a discrete version of eq. 3 is solved by applying the Baranov catch equation across ages (Quinn and Deriso 1999). Here that is not possible because of the continuous age structure and selectivity. Instead, we

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solve eq. 3 by numerical integration, feasible after specifying a selectivity function.

We use a logistic model of selectivity, chosen for its ability to depict a suite of selectivity patterns:

$$(4) \quad s(a) = \frac{1}{1+\exp[-\eta_s(a-a_1)]}$$

Parameter η_s is the slope of the selectivity function, which controls the rate that selectivity approaches full selection (i.e., $s(a) = 1$) with age. Parameter a_1 is an inflection point that controls where $s(a) = 0.5$. In an age-structured population, selectivity relative to maturation can strongly influence F_{MSY}. We therefore express a_1 in terms of the age at maturity (a_{MAT}) shifted by a scaling parameter (τ) such that $a_1 = \tau a_{MAT}$. The parameter τ serves as a focus for analyzing the BRPs with respect to the timing of selection relative to age at maturity. For logistic selectivity, the solution to the integral of eq. 2 is

$$(5) \quad \int_0^a s(x)\mathrm{d}x = \frac{-\eta_s a' + \log(1+\exp[\eta_s a'\,]) + \eta_s a'' - \log(1+\exp[\eta_s a''\,])}{\eta_s}$$

where $a' = \tau a_{MAT} - a - 0.5$ and $a'' = \tau a_{MAT} - a + 0.5$ (Beyer 1978).

The age at maturity is defined here as the age when 50% of the individuals are mature. The proportion of mature individuals in a cohort $m(a)$ also follows the logistic model

$$(6) \quad m(a) = \frac{1}{1+\exp[-\eta_m(a-a_{MAT})]}$$

where η_m controls the rate of maturation.

The length (L) of survivors increases with age according to the von Bertalanffy growth equation (von Bertalanffy 1938):

$$(7) \quad L(a) = L_\infty[1-\exp(-Ka)]$$

where K is the Brody growth coefficient and L_∞ is the asymptotic length. We assume for simplicity that $L(0) = 0$ and that weight at age is proportional to length cubed ($W(a) \propto L(a)^3$). Hence the spawning stock biomass (SSB) is

$$(8) \quad \mathrm{SSB} = \int_a N(a)m(a)W(a)\mathrm{d}a$$

The number of fish at age 0 in a cohort (N_0) is determined by equilibrium recruitment (R_{eq}) from a Beverton–Holt or Ricker stock–recruit function. Following convention, we parameterize the stock–recruit functions in terms of the level of unfished recruitment (R_0) and steepness (h). The parameter R_0 is the recruitment that occurs at the carrying capacity, defined by the intersection of the stock–recruit curve and the replacement line. Replacement occurs where recruitment exactly supplants the spawning biomass (i.e., line with slope equal to ϕ_0, the spawning biomass per recruit at $F = 0$). The Beverton–Holt parameterization is

$$(9) \quad R = \frac{0.8R_0h\,\mathrm{SSB}}{0.2\phi_0R_0(1-h)+(h-0.2)\mathrm{SSB}}$$

where R is recruitment. Similarly, the Ricker parameterization is

$$(10) \quad R = \frac{\mathrm{SSB}}{\phi_0}\exp\left[h'\left(1-\frac{\mathrm{SSB}}{R_0\phi_0}\right)\right]$$

A standard assumption in fishery modeling, adopted here, is that all density dependence in population dynamics can be described by the stock–recruit function.

In eq. 9, h refers to the proportion of R_0 that would be produced by 20% of the unfished spawning biomass (SSB_0; i.e., the carrying capacity) and ranges from 0.2 to 1.0 (Mace and Doonan 1988; Haddon 2001; Myers et al. 2002). In the Ricker stock–recruit function of eq. 10, h' does not have the same meaning because maximum recruitment may exceed R_0 and therefore h' ranges from zero to infinity (Kimura 1988; Dorn 2002). Under equilibrium conditions and assuming that $R_0 = K$, the Beverton–Holt and Ricker steepness parameters are related to the maximum reproductive rate λ and the intrinsic growth rate r (Quinn and Deriso 1999; McAllister et al. 2001; Myers et al. 2002),

$$(11) \quad \lambda = \exp(r) = \frac{4h}{(1-h)} = \exp(h')$$

We note that the "4" in eq. 11 is a result of defining h with the arbitrary choice of 20% of SSB_0. It could be eliminated if h were redefined using 50% of SSB_0, which would result in more elegant solutions of steepness' relationships to other population parameters (Kimura 1988).

Using the convention of 20%, the equilibrium recruitment R_{eq} corresponding to a given F is

$$(12) \quad R_{eq} = R_0\left[\frac{0.8h\,\mathrm{SPR}-0.2(1-h)}{\mathrm{SPR}(h-0.2)}\right]$$

for the Beverton–Holt function. For the Ricker function, the analogous equation is

$$(13) \quad R_{eq} = \frac{R_0}{\mathrm{SPR}}\left(1+\frac{\log \mathrm{SPR}}{h'}\right)$$

In eqs. 12 and 13, SPR is the spawning potential ratio defined previously.

Life-history invariants

The above model contains three biological parameters that drive, and are shaped by, evolution: natural mortality M, the growth coefficient K, and age at maturity a_{MAT}. Correlations among these parameters are well documented in comparative life histories (Stearns 1992; Charnov 1993). As the result of trade-offs between reproduction and survival, species with relatively high natural mortality tend to grow quicker and mature younger; conversely, species that devote less energy toward growth and gonad development tend to suffer lower natural mortality (Roff 1984; Jensen 1996). Although many different life-history strategies exist, certain combinations of life-history parameters can be expressed as dimensionless quantities that have been shown to be invariant across a wide range of taxa (Charnov 1993).

One such dimensionless quantity is M/K. Charnov (1993) analyzed the relationship between M and K by applying or-

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dinary regression to a data set (Pauly 1980) of 175 fish stocks from around the world representing 40 families and 110 species. The data display broad scatter, but Charnov (1993) found the mean relationship to be nearly constant:

$$M/K \cong 1.65 \quad (14)$$

Beverton (1992) provided a theoretical relationship among life-history parameters M, K, and a_{MAT} based on the dimensionless ratio of length at maturity to asymptotic length. Beverton (1992) reported two expressions for that ratio: $L(a_{MAT})/L_\infty = 3/(3 + M/K)$ from Holt (1958) and $L(a_{MAT})/L_\infty = 1 - \exp(-Ka_{MAT})$, the ratio expected from von Bertalanffy growth. By equating the right-hand sides of the two expressions, one can solve for a_{MAT} as a function of M and K:

$$a_{MAT} = \log\frac{\left(\frac{3K + M}{M}\right)}{K} \quad (15)$$

Equation 15 requires the assumption that age at maturity occurs so as to maximize lifetime egg production (Beverton 1992).

Analyses

To analyze the three BRPs for relevance to many fisheries, we apply the model over a broad range of biological and selectivity patterns. For each combination of parameters, we use Shepherd's method to determine F_{MSY} (Shepherd 1982). The method searches iteratively for a solution of F that maximizes yield (eq. 3) under a given stock–recruit curve and a stable age structure. Catch, numbers, and spawning biomass are computed over ages 0 to a maximum defined as the age when a negligible percent (1×10^{-6}) of the cohort survives. Once deemed a target, F_{MSY} is now more often considered a limit reference point (Mace 2001). Based on $F = F_{MSY}$, we compute corresponding values of the three BRPs (i.e., c_1, c_2, and c_3). In actual fishery management, the method is reversed: c_i's are set first and the corresponding F serves as a proxy for the unknown F_{MSY}. Our analyses identify the BRP values where such proxies are reliable.

In our analyses, we systematically vary biological parameters of steepness (h or h') and growth (K). For each value of K, the life-history invariant relationships (eqs. 14 and 15) provide values of M and a_{MAT}. We also systematically vary fishery parameters controlling the selectivity pattern (η_s) and the timing of selection relative to age at maturity (τ). Parameter η_s takes on three different values. An extreme value ($\eta_s = \infty$) represents selectivity that changes from zero to one almost instantaneously with age ("knife-edged" selection). A low value ($\eta_s = 0.5$) represents selectivity that increases slowly over a wide range of ages. A moderate value ($\eta_s = 3.0$) represents an intermediate selectivity. We assume that selectivity increases with age at the same rate as maturation ($\eta_s = \eta_m$), justified by noting that selectivity as it relates to availability and maturation are typically linked by migration patterns or size-at-age distributions. As with η_s, τ takes on three different values. The values represent selection before ($\tau = 0.75$), at ($\tau = 1.0$), or after ($\tau = 1.25$) the age when 50% of the cohort is mature. For all combinations of biological and fishery parameters, we examine values of and relationships between the three BRPs at $F = F_{MSY}$.

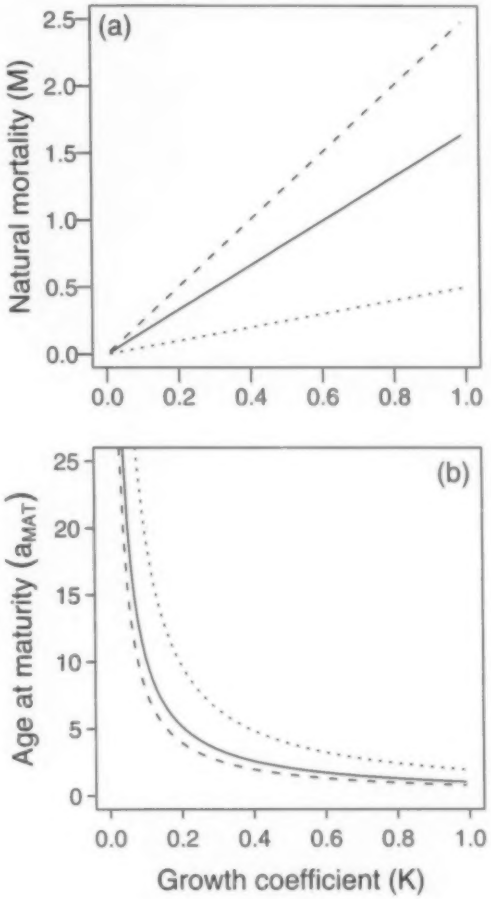

Fig. 1. Relationships of (*a*) natural mortality M and (*b*) age at maturity a_{MAT} to the von Bertalanffy growth parameter K using three values of M/K: 2.5 (broken line), 1.65 (solid line), and 0.5 (dotted line).

A key consideration is how sensitive the above results are to the underlying M/K ratio. Charnov's fitted ratio (Charnov 1993) of $M/K = 1.65$ describes the "average stock", but this does not necessarily fit well for any given stock. Depending on the estimation scheme, Charnov (1993) reported a M/K range of 1.6 to 2.1. Beverton (1992) suggested a lower bound closer to 0.5, based on a different data set. We conservatively consider wide deviation from the "average stock", using a low ratio of $M/K = 0.5$ and a high ratio of $M/K = 2.5$ (Fig. 1*a*). Such a range should approximate the gamut of plausible M/K ratios, and it creates a corresponding range of values for age at maturity (Fig. 1*b*).

Results

The use of life-history invariants allows each BRP to be viewed as a function of the growth coefficient (K) and steepness (h or h'). The values from the M-based policy are illustrated in Fig. 2. In the case of knife-edged selection and

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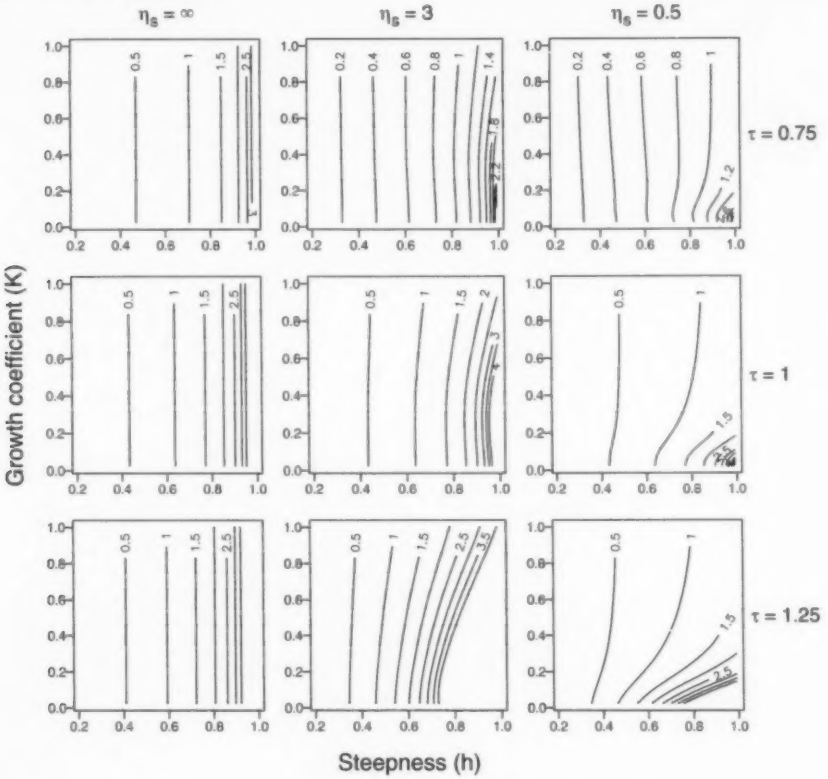
Fig. 2. Values of the *M*-based biological reference point (up to 4.0 only) using the Beverton–Holt stock–recruit function and an *M*/*K* ratio of 1.65. Rows correspond to values of τ, the ratio of the age at 50% selectivity to the age at 50% maturity, and columns correspond to values of η_s, the slope of selectivity.



maturity, the *M*-based BRP depends only on steepness (Fig. 2, left column). However, with a shallower slope in the selection curves, the BRP depends on both *K* and *h* nonlinearly, particularly at higher levels of steepness (Fig. 2). For any given slope, the shift (τ) in selection relative to maturity affects the BRP value. As τ increases, the BRP tends to increase for similar values of steepness (Fig. 2).

The *B*-based and SPR BRPs exhibit patterns similar to those of the *M*-based BRP: knife-edged selection results in BRP values that are independent of *K*, and shallower-sloped selection results in values that depend nonlinearly on both *K* and *h* (Figs. 3 and 4). However, unlike the *M*-based BRP, the *B*-based and SPR BRPs are hardly influenced by τ. With increased τ, there is no change in either BRP for steepness smaller than ~0.7, and only a slight reduction otherwise (Figs. 3 and 4).

The analyses show that among the three BRPs, the *M*-based policy depends most on fishery and life-history parameters, whereas SPR depends least (Figs. 2, 3, and 4). Only at the highest steepness values (>0.7) does SPR begin to depend on life-history parameters (*M*, *K*, a_{MAT}) represented by *K* (Fig. 4). However, all three BRPs are highly dependent on steepness (Figs. 2, 3, and 4).

All three BRPs under the Ricker stock–recruit function (not shown) exhibit qualitatively similar patterns as under the Beverton–Holt. SPR under the Ricker is highly dependent on steepness and nearly independent of the life-history parameters summarized by *K*. Again, there is some dependence on *K* at the highest steepness values, but a weaker dependence under the Ricker function than the Beverton–Holt.

Plotting the BRPs against one another, over the full range of fishery and life-history parameters, reveals their interrelationships (Fig. 5). For each selectivity pattern, the *B*-based and SPR BRPs have a tight relationship, a result of their independence from life history. The relationships are not nearly as tight between the *M*-based BRP and either of the other two, particularly for selectivities other than knife-edged. These results indicate that the *B*-based and SPR BRPs are compatible over a broad range of fishery and life-history parameters, whereas neither is so compatible with the *M*-based policy.

The relationship between the *B*-based and SPR BRPs appears to be quite strong (Fig. 5); however, that result is limited to the Beverton–Holt stock–recruit function and a single value of *M*/*K* = 1.65. A fuller examination using both the Beverton–Holt and Ricker functions and several *M*/*K* ratios indicates more variability (Fig. 6). Nonetheless, the relationship remains fairly consistent for each stock–recruit function, particularly in the case of knife-edged selection. Other than for the highest BRP values, the relationship depends

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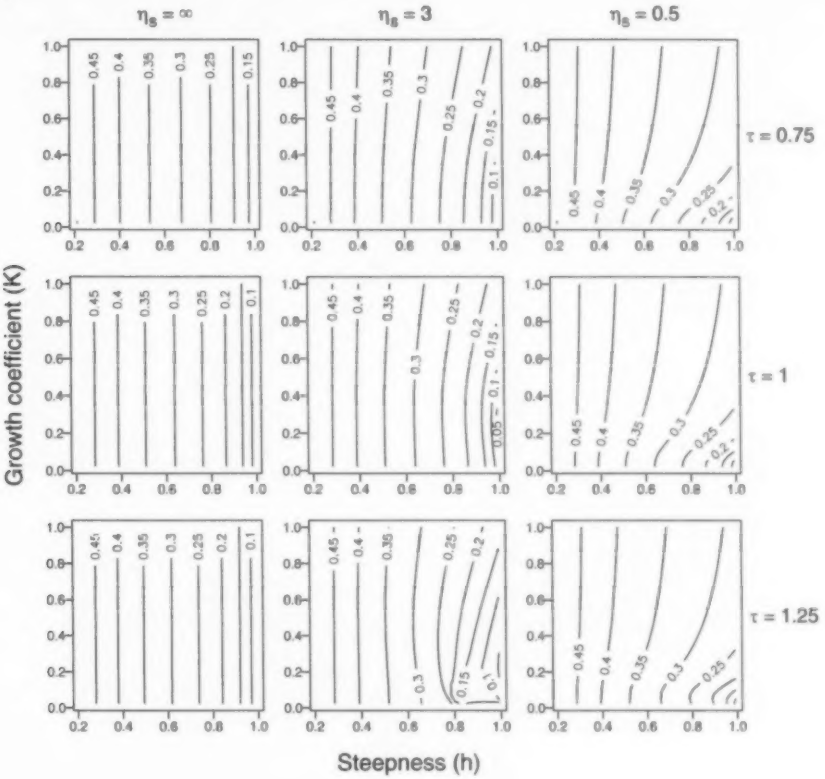
Fig. 3. Values of the *B*-based biological reference point using the Beverton–Holt stock–recruit function and an *M*/*K* ratio of 1.65. Rows correspond to values of τ, the ratio of the age at 50% selectivity to the age at 50% maturity, and columns correspond to values of η_s, the slope of selectivity.



considerably on the underlying stock–recruit function. Given similar population parameters, the Ricker function tends to produce higher *B*-based values and lower SPR values than does the Beverton–Holt function. As suggested in Fig. 6, the commonly assumed SPR proxy of $F_{40\%}$ would result in SSB levels near 30 and 40% of unfished levels for the Beverton–Holt and Ricker functions, respectively.

The BRP values can be fairly sensitive to the *M*/*K* ratio, shown here for the selectivity pattern with intermediate slope (Fig. 7). By using low and high extremes of the *M*/*K* ratio, Fig. 7 presents the full variability in BRP values due to deviations from the expected life histories. The *M*-based values show the greatest sensitivity to deviations in *M*/*K* for both Beverton–Holt and Ricker stock–recruit functions. The *B*-based values appear moderately sensitive, with greater variability under the Ricker function than under Beverton–Holt. The SPR values are by far the most robust to deviations in *M*/*K* for both stock–recruit functions.

The BRP values are more sensitive to deviations in *M*/*K* when the selectivity pattern has a shallower slope (not shown). When selectivity is knife-edged, the values change little or not at all with deviations in the *M*/*K* ratio (Fig. 8). Again, SPR is the most robust, with the *B*-based BRP a close second. Furthermore, the previous result that τ does not affect SPR values holds across the full range of *M*/*K* ratios.

Scaling the Ricker steepness (h') to the Beverton–Holt steepness (h) elucidates how choice of the stock–recruit function affects the BRPs (Fig. 9). For each BRP, values under the two functions are nearly identical at low steepness and diverge as steepness increases. For a given steepness, the Beverton–Holt function produces higher SPR values and lower *B*- and *M*-based values. The management implication is that for a given population growth rate, a stock with Ricker recruitment can support a higher rate of exploitation than can one with Beverton–Holt recruitment.

Discussion

We have used a population model based on invariant life-history relationships to analyze common fish harvest policies. Life-history invariants have a solid theoretical basis (Roff 1984; Beverton 1992; Charnov 1993) and have been valuable in other fishery applications (Mangel 1996; Charnov and Skuladottir 2000; Frisk et al. 2001). Our use of them has allowed insights to emerge about biological reference points, particularly regarding their relations to life-history parameters and each other. Many of these insights carry strong implications for management.

Previous studies have drawn connections between BRPs and life-history parameters. Mace (1994) examined several

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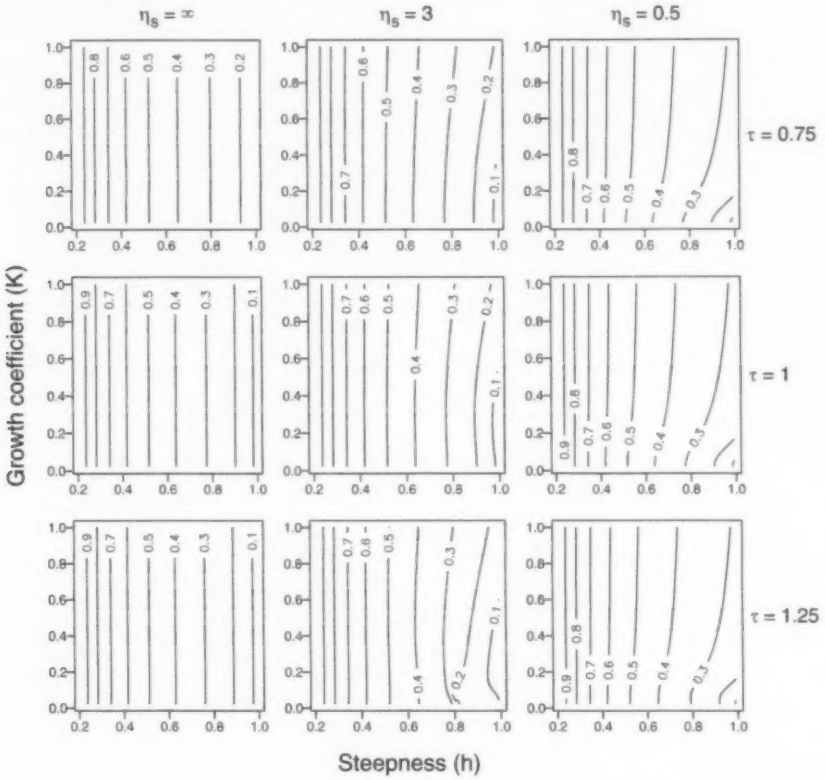
Fig. 4. Values of the spawning potential ratio, SPR, biological reference point using the Beverton–Holt stock–recruit function and an M/K ratio of 1.65. Rows correspond to values of τ, the ratio of the age at 50% selectivity to the age at 50% maturity, and columns correspond to values of η_s, the slope of selectivity.



BRPs as a function of steepness, using knife-edged maturity, coincident knife-edged recruitment, and three different values each for M, K, and a_{MAT}. Clark (1991) considered shallower-sloped schedules in maturity and recruitment and the possibility of their offset using life-history parameters typical of New England groundfish. Goodyear (1993) analyzed SPR with life-history parameters describing Gulf of Mexico red snapper. Our study broadens the results from those previous by considering a full range of life-history parameters, the complete spectrum of steepness values, and an array of maturity and recruitment patterns. The analysis confirms that there is no single BRP value that can safely be applied to all stocks, a result that is only recently gaining consensus (Clark 2002; Dorn 2002; Myers et al. 2002).

The three reference points analyzed here, M based, B based, and SPR, do not constitute an exhaustive list. Even so, the methods here are general and could be used to investigate relationships between virtually any BRPs. Two others often used are F_{max} and $F_{0.1}$ (Sissenwine and Shepherd 1987). F_{max} is the fishing mortality rate that maximizes yield per recruit, and $F_{0.1}$ is the fishing mortality rate when the yield per recruit function has a slope of 10% of that at the origin. $F_{0.1}$ was originally proposed to be a more ecologically conservative reference than F_{max}, without too much sacrifice in yield (Gulland and Boerema 1973). However, both ignore effects on the spawning population, and this can be dangerous (Quinn and Deriso 1999).

Of this paper's three BRPs, the M-based policy is the most sensitive to model parameters. Its applicability is limited to when the stock–recruit function is well determined and life history and steepness parameters are well estimated. Such conditions are rarely met, if ever. Natural mortality itself is particularly difficult to estimate accurately. In addition, the M-based BRP, like $F_{0.1}$ and F_{max}, ignores effects on spawning stock size. We recommend that M-based policies be eliminated from fishery management altogether.

The B-based and SPR BRPs do account for effects on the spawning population. Relative to the M-based policy, both are more robust to misspecifications in life-history parameters. The SPR BRP is nearly invariant with respect to life-history parameters M, K, and a_{MAT}. For these reasons, we advocate SPR as the BRP most likely to achieve management goals when life-history data are limited.

The results indicate potential inconsistencies in some harvest policies. For example, in the case of West Coast groundfish, the current proxy for B_{MSY} is a B-based proxy, $B_{40\%}$, defined as 40% of the unfished biomass size. Current management proxies for F_{MSY} for West Coast groundfish rely on SPR values ranging from $F_{40\%}$ to $F_{50\%}$, depending on the species group (PFMC 2001). Based on our analyses compar-

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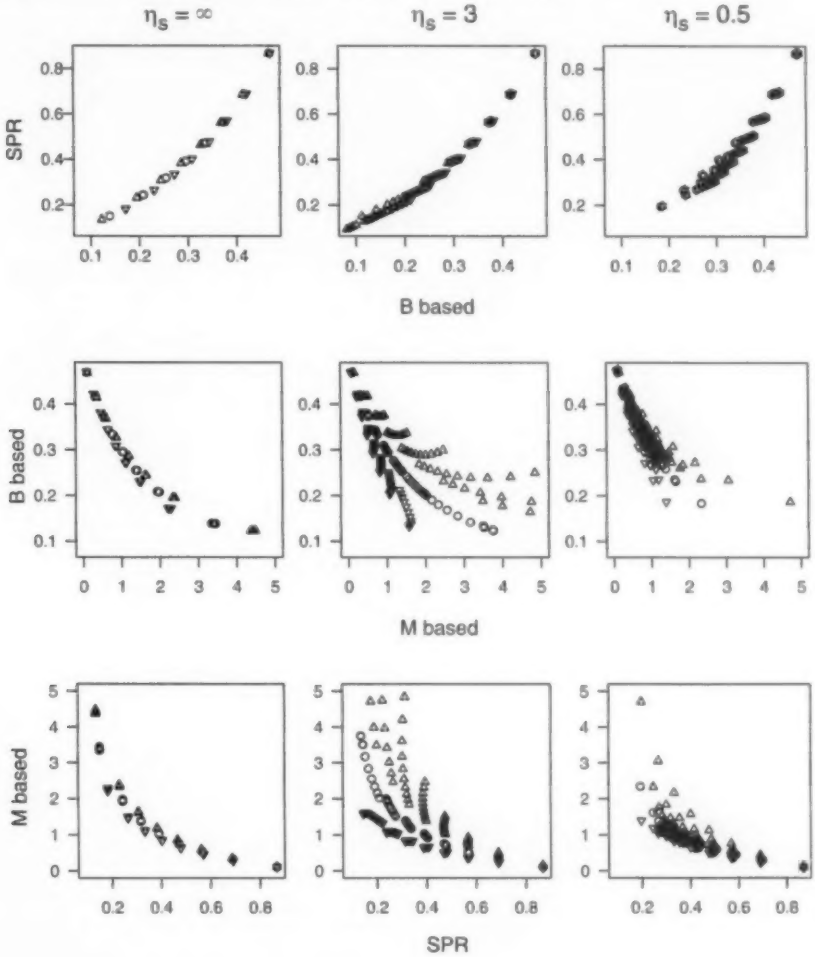
Fig. 5. Interrelationships of the *M*-based, *B*-based, and spawning potential ratio, SPR, biological reference points using the Beverton–Holt stock–recruit function and an *M*/*K* ratio of 1.65. Symbols refer to the value of τ, the ratio of the age when a cohort reaches 50% selectivity to the age at 50% maturity: τ = 0.75, inverted triangle; τ = 1.0, circle; and τ = 1.25, triangle. For each value of τ, reference points are calculated for all combinations of steepness (incremented from 0.2 to 1.0) and the von Bertalanffy growth parameter (incremented from 0.1 to 1.0). Columns correspond to values of η_s, the slope of selectivity.



ing BRP values with each other, $B_{40\%}$ equates to SPR values of roughly $F_{65\%}$ and $F_{30\%}$ for the Beverton–Holt and Ricker stock–recruit functions, respectively. In either case, the proxies appear inconsistent if either function is assumed to underlie the population dynamics.

This study reiterates a fundamental message in fishery management: understanding the relationship between stock and recruitment is crucial. Very different BRP values emerge under the Beverton–Holt and Ricker functions, particularly for higher steepness values. With typical stock–recruit data, distinguishing between the two functions is no easy task. Judgment is often relegated to statistical software and based on which function provides a better fit to the data. In many data sets, those fits are driven by only a small number of data points with high stock size, where variability in recruitment is greatest. In such cases, distinction between the Beverton–Holt and Ricker functions relies heavily on chance. This is risky given the disparate management implications stemming from the two functions. A better approach, we believe, is to choose a stock–recruit function a priori based on the presence or absence of biological mechanisms assumed by the model. Four examples of mechanisms that can lead to the dome shape of the Ricker function are adult cannibalism on juveniles, density-dependent disease transmission, density-dependent quality of spawning sites, and density-dependent growth accompanied by size-dependent mortality (Hilborn and Walters 1992). Unless good evidence supports a plausible mechanism for the Ricker function, the Beverton–Holt function is likely a better choice for management, especially given its more conservative BRP values.

In our analysis, we have assumed deterministic recruitment and survival. Other studies have examined the effects

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Fig. 6. Interrelationships of the *B*-based and spawning potential ratio, SPR, biological reference points using the Beverton–Holt (solid) and Ricker (shaded) stock–recruit functions for various values of η_s, the slope of selectivity. For each panel, analyses use a range of values for K (0.025–1.0), M/K (0.5, 1.65, 2.5), and τ (0.75, 1.0, 1.25).

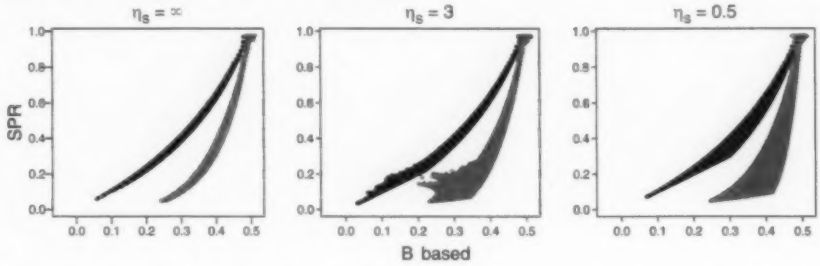
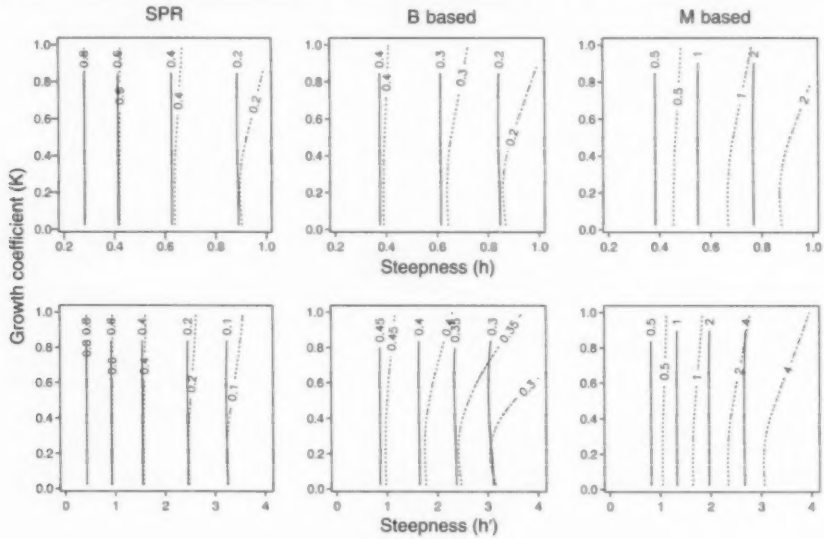


Fig. 7. Sensitivity of the spawning potential ratio (SPR), *B*-based, and *M*-based biological reference points to the M/K ratio under the Beverton–Holt (top row) and Ricker (bottom row) stock–recruit functions. Analyses use low (0.5, dotted line) and high (2.5, solid line) extremes of the M/K ratio. Other model specifications set at their intermediate values ($\tau = 1$ and $\eta_s = 3$).



of stochasticity on structured population models. Clark (1993) extended a previous study (Clark 1991) to include stochastic and serially correlated recruitment, resulting in higher recommended SPR. Tuljapurkar (1990) provided an approximation of the population growth rate for stochastic matrix models and noted that stochasticity often decreases the growth rate. Given those results, it is likely that by adding stochasticity to our analysis, similar BRP values would require higher steepness values. It is also likely that the relationship between BRPs and steepness would depend on the details of implementing stochasticity, such as the type(s) of random noise and correlation structure among parameters. Further research into the overall effects of stochasticity on BRPs is needed.

Ultimately, effective fishery management relies on obtaining some metric of the population growth rate. Steepness is a useful concept because it relates directly to population growth. It measures the maximum reproductive rate and, hence, the resilience of a stock to exploitation. Furthermore, steepness is comparable across stocks because it is independent of selectivity, unlike F_{MSY}. Not surprisingly, all three BRPs are highly dependent on steepness. Our analysis can provide some understanding of the population growth rate or steepness implied when choosing an SPR proxy for management. Choosing a value of any BRP without regard to steepness would likely result in faulty management, and this implies that accurate estimation of steepness should be a top-priority goal. We recommend that BRPs be developed based on explicit assumptions about steepness.

How can steepness best be estimated when the stock–recruitment time series is of limited duration? The answer is not obvious, but carefully designed experiments and meta-analyses offer enticing possibilities (Myers and Barrowman 1996; Myers 2001). The optimal experimental design should, at a minimum, achieve reliable information about recruitment at high (virgin) and low (20% of virgin) SSB. Meta-analysis may be more feasible, averting the problem of a short time series by, in effect, substituting space for time. Multiple time series of spatially separated stocks, perhaps from different species with similar life histories, could be

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Fig. 8. Values of biological reference points under the Beverton–Holt stock–recruit function and "knife-edged" selectivity, plotted for a range of steepness and *M*/*K* ratios. Columns correspond to values of τ, the ratio of the age at 50% selectivity to the age at 50% maturity.

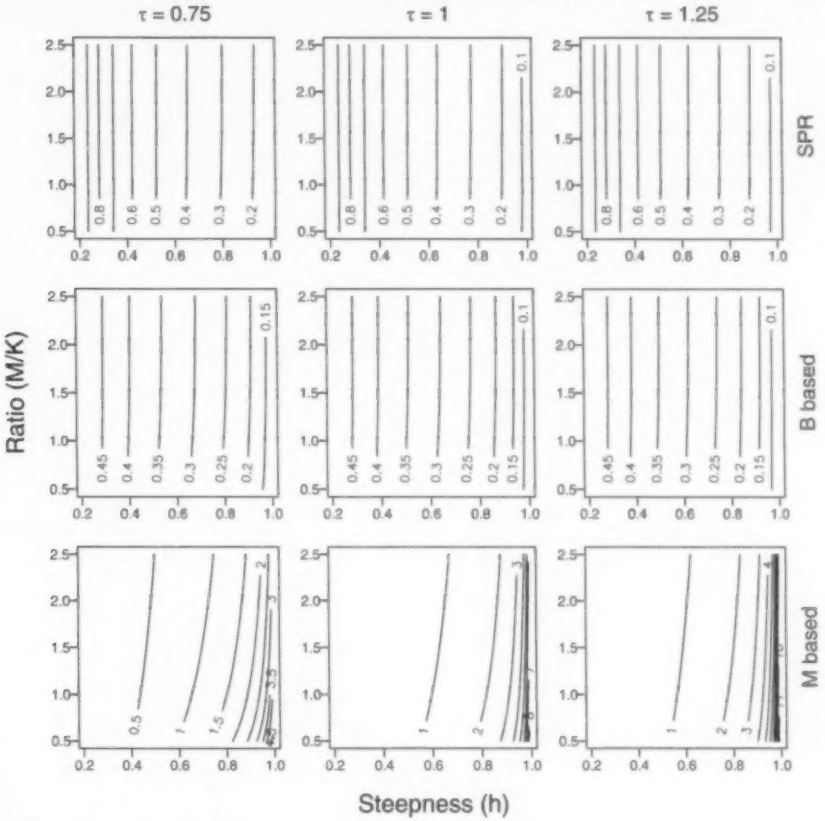
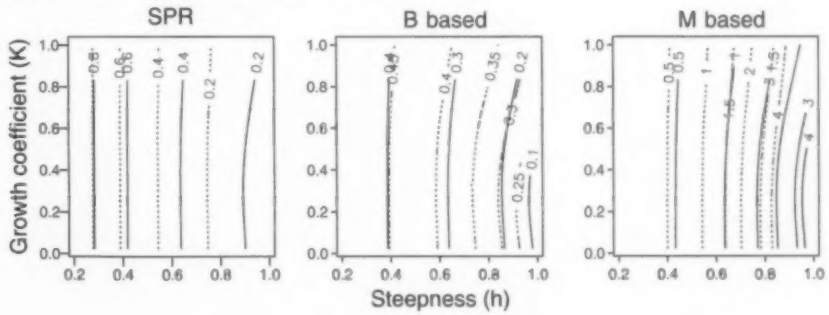


Fig. 9. Comparison of the spawning potential ratio (SPR), *B*-based, and *M*-based biological reference points under the Beverton–Holt (solid line) and Ricker (dotted line) stock–recruit functions. Ricker steepness is scaled to Beverton–Holt steepness by eq. 11. Other model specifications are set at their intermediate values (τ = 1 and η_s = 3).



used as a surrogate for a single, longer time series. At the least, such an approach could be used to generate prior distributions of steepness for Bayesian analyses (McAllister et al. 2001; Myers et al. 2002). Distributions of steepness could also be used as input for the model here to generate probability distributions of BRPs.

Meta-analyses may also reveal correlations of steepness with biological and environmental factors. Myers et al. (2002) examined several potential covariates and found a weak negative relationship between steepness and natural mortality, a seemingly contradictory relationship. As fisheries are depleted and rebuilt and stock–recruit databases improve, future meta-analyses may find stronger biological correlations on which to base management. However, further analyses may also reveal the opposite: that population growth rates for some stocks, as measured through steepness, are so strongly

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linked to environmental and ecological conditions that management is better off acting on information from surveys of year-class strength than on BRPs assuming equilibrium conditions.

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Relationships among nutrients, phytoplankton, macrophytes, and fish in prairie wetlands

Kyle D. Zimmer, Mark A. Hanson, and Malcolm G. Butler

Abstract: Phytoplankton abundance and nutrient concentrations in shallow-water ecosystems are influenced by submerged macrophytes, zooplankton, and fish, but few studies have simultaneously assessed the influence of all three variables. We sampled 18 semipermanent prairie wetlands for 5 years to assess influences of minnows, submersed macrophytes, cladocerans, and drainage history on phytoplankton abundance and concentrations of nitrogen (N) and phosphorus (P). Our macrophyte data reflect the abundance of three distinct species assemblages (*Chara*, *Potamogeton*, and *Myriophyllum* assemblages) typical of these wetlands. Partial redundancy analysis showed only the *Chara* and *Potamogeton* assemblages and fish to be significantly related to algal abundance, N, and P. Macrophytes and fish together explained 40% of the total variance, but the *Chara* assemblage explained threefold, and the *Potamogeton* assemblage twofold, more variance than did presence/absence of fish. However, relationships with N and P differed for the two groups of macrophytes: P showed a strong negative relationship with both plant assemblages, and N showed a weak negative relationship with *Chara* but no relationship with *Potamogetons*. Our results indicate that phytoplankton and nutrient concentrations in prairie wetlands are strongly influenced by submersed macrophytes, although influences may depend on plant community composition.

Résumé : Dans les écosystèmes aquatiques peu profonds, l'abondance du phytoplancton et les concentrations des nutriments subissent l'influence des macrophytes submergés, du zooplancton et des poissons; peu d'études, cependant, ont mesuré les effets simultanés des trois variables. Notre échantillonnage de 5 ans qui couvre 18 terres humides semi-permanentes des prairies nous a permis d'évaluer les effets des ménés, des macrophytes submergés, des cladocères et de l'histoire du bassin versant sur l'abondance du phytoplancton et les concentrations d'azote (N) et de phosphore (P). Nos données sur les macrophytes présentent les abondances des trois regroupements d'espèces distincts (les regroupements de *Chara*, de *Potamogeton* et de *Myriophyllum*) typiques de ces terres humides. Une analyse de redondance partielle révèle que seuls les regroupements de *Chara* et de *Potamogeton* et les poissons montrent une relation significative avec la biomasse des algues, N et P. Les macrophytes et les poissons expliquent ensemble 40 % de la variance totale, mais le regroupement de *Chara* et celui de *Potamogeton* expliquent respectivement trois et deux fois plus de variance que la présence ou absence de poissons. Cependant, les relations des deux groupes de macrophytes avec N et P sont différentes. P montre une forte relation négative avec les deux groupements de plantes et N a une faible relation négative avec *Chara*, mais aucune relation avec *Potamogeton*. Nos résultats démontrent que, dans les terres humides des prairies, le phytoplancton et les concentrations de nutriments sont fortement influencés par les macrophytes submergés, bien que les effets puissent varier d'après la composition de la communauté de plantes.

[Traduit par la Rédaction]

Introduction

Phytoplankton abundance and concentrations of water-column phosphorus (P) and nitrogen (N) are extremely variable and dynamic in shallow lakes and wetlands and may reach very high concentrations in many ecosystems (Hanson and Butler 1994; DeMott and Gulati 1999; Zimmer et al. 2002). Understanding the mechanisms responsible for this variance is important, as phytoplankton abundance and water clarity exert ecosystem-scale influences. Many studies have shown that shallow lakes commonly exist in one of two alternative stable states: either a clear-water, macrophyte-dominated state or a turbid-water, phytoplankton-dominated state (reviewed by Scheffer 1998). Phytoplankton abundance plays a key role in the stable-state model through effects on water clarity, which in turn influences the abundance of submersed macrophytes. Although ecosystem-scale influences of water clarity are driven directly by phytoplankton abun-

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K.D. Zimmer[1,2] **and M.G. Butler.** Department of Biological Sciences, North Dakota State University, Fargo, ND 58105, U.S.A.
M.A. Hanson. Wetland Wildlife and Populations Research Group, Minnesota Department of Natural Resources, Bemidji, MN 56601, U.S.A.

[1]Corresponding author (email: KDZIMMER@Stthomas.edu).

[2]Present address: Department of Biology, University of St. Thomas, Mail #OWS390, 2115 Summit Ave., St. Paul, MN 55105, U.S.A.

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dance, algal abundance may be constrained by the availability of nutrients (Blindow et al. 2000). Thus, identifying factors that influence phytoplankton abundance require identification of factors regulating N and P dynamics.

Many factors influence phytoplankton abundance and N and P concentrations in shallow lakes and wetlands, but biotic interactions are thought to be especially important (Hanson and Butler 1994; Jeppesen et al. 1997). More specifically, presence/absence of fish, abundance of submersed macrophytes, and abundance of zooplankton have been shown to have particularly strong influences in these systems (Meijer et al. 1999; Blindow et al. 2000; Zimmer et al. 2002). However, interactions among fish, macrophytes, and zooplankton are complex, and the extent to which they influence water-quality characteristics appears to vary widely (Stephen et al. 1998; Blindow et al. 2000). Thus, a clear understanding of nutrient and phytoplankton dynamics necessitates consideration of all of these variables. At present, few studies have simultaneously assessed the relative importance of zooplankton, macrophytes, and fish on water-quality characteristics at the ecosystem scale. This is especially true in wetlands of the Prairie Pothole Region (PPR) of North America.

Phytoplankton abundance and concentrations of N and P are highly variable among PPR wetlands (Zimmer et al. 2002), but specific causal mechanisms are unclear. Similar to other shallow-water systems, phytoplankton biomass and nutrient concentrations reflect presence or absence of fish and the abundance of submersed macrophytes and zooplankton, all of which vary widely among prairie wetlands (Hanson and Riggs 1995; Zimmer et al. 2002; K.D. Zimmer, M.A. Hanson, and M.G. Butler, unpublished data). Fathead minnows (*Pimephales promelas*) are often the dominant fish species in terms of numbers and biomass in PPR wetlands, but their presence and abundance vary among wetland basins (Hanson and Riggs 1995; Zimmer et al. 2000). These fish may have strong influences on PPR wetlands, as they are often associated with reduced abundances of submersed macrophytes and zooplankton (Hanson and Riggs 1995; Zimmer et al. 2000). Additionally, Zimmer et al. (2002) reported that chlorophyll *a* concentrations averaged fivefold higher in wetlands with minnows relative to comparable fishless sites. However, it is difficult to identify specific mechanisms responsible for the differences in phytoplankton abundance reported by Zimmer et al. (2002), given the widespread natural variation in wetland characteristics and distinct differences between wetlands with and without minnows. Higher abundance of phytoplankton in wetlands with fish may reflect direct influences of fish (i.e., nutrient excretion), reduced zooplankton abundance, reduced abundance of submersed macrophytes, or various combinations of cumulative effects. Assessing the relative importance of these variables seems especially important as Zimmer et al. (2002) reported consistent fish effects on algal biomass, but no differences in concentrations of N and P between wetlands with and without fish. However, concentrations of P were higher in restored wetlands compared with wetlands that had never been drained (Zimmer et al. 2002).

Here, we examine phytoplankton abundance and nutrient concentrations (dependent variables) in the same wetlands used in Zimmer et al. (2002) and K.D. Zimmer, M.A. Hanson, and M.G. Butler (unpublished data) and assess the relative importance of drainage history of the wetlands (restored versus nondrained), presence/absence of minnows, abundance of submersed macrophytes, and abundance of zooplankton (independent variables). Our analysis represents a unique opportunity to discern the specific influence of each independent variable on nutrient dynamics and phytoplankton abundance in PPR wetlands, while also considering the influence of the other independent variables. Our specific goals were (*i*) to examine relationships among the dependent variables, (*ii*) to examine relationships among the independent variables, (*iii*) to identify independent variables that explained significant variation in the dependent variables, and (*iv*) to examine specific relationships between individual dependent and independent variables.

Methods

Study sites

From 1995 to 1996, study wetlands were selected from a population of candidate sites on federal and private lands within the PPR of west-central Minnesota. Ten restored, semipermanent wetlands were selected based on criteria including presence/absence of fathead minnows, age-since-restoration (5–10 years), adjacent upland cover type and associated land use, and similarities in hydroperiod. Five wetlands with minnow populations were chosen along with five that were fishless, based on sampling during spring of 1996. Ten natural wetlands (nondrained basins) were also selected based on similar criteria, again five with and five without minnow populations. Study wetlands were dispersed across 3751 km^2 in west-central Minnesota, and the uplands of all sites were vegetated mainly by prairie grasses. Of the original 20 wetlands, one site (nondrained, fishless) was colonized by fathead minnows and many ecosystem characteristics changed dramatically in response to this event (see Zimmer et al. 2001). Another wetland (restored, with fish) was drained for management purposes during 2000. Incomplete data sets for these two sites would have confounded our multivariate procedures, so we restricted our analyses to the remaining 18 wetlands only.

Field sampling

The study sites were sampled during each growing season from 1996 through 2001. A water sample was collected from the center of each wetland every 3 weeks from May through August in each of the five study years, for a total of six sampling dates per year. Each sample was analyzed for total phosphorus and total nitrogen in the water column (hereafter P and N, respectively) and chlorophyll *a* (American Public Health Association (APHA) 1995). Turbidity was also measured in the field on the same dates using a portable nephelometer. We then determined the seasonal means of chlorophyll *a*, P, N, and turbidity for each wetland in each year, and we calculated seasonal mean N:P (by mass) for each wetland based on the seasonal means of N and P. These seasonal means were used for all statistical analyses. Seasonal means of the dependent variables averaged across the five years are summarized in Table 1.

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Table 1. Summary statistics of chlorophyll *a*, total phosphorus (P), total nitrogen (N), total nitrogen – total phosphorus ratio (N:P), and turbidity (nephelometric turbidity units, NTUs) in the 18 study sites during the five years of the study.

Response variable	Mean	Standard deviation	Range
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	25.7	28.7	2.1–151.8
P ($mg \cdot L^{-1}$)	0.11	0.08	0.02–0.35
N ($mg \cdot L^{-1}$)	1.9	0.6	0.7–3.7
N:P (mass)	24.3	17.6	5.0–112.3
Turbidity (NTUs)	7.1	6.0	1.2–27.1

Note: Statistics were calculated from the seasonal mean in each wetland in each year. All multivariate analyses were conducted on chlorophyll *a*, P, and N only; N:P and turbidity were treated as supplementary response variables.

The relative abundance of submersed macrophytes was assessed in August of each year using techniques modified from Depp and Lathrop (1992). Five transects radiating from the center of the basin to the wetland edge were randomly selected each year in each wetland. Three stations for sampling macrophytes were established along each transect: (1) an emergent station located at the interface of emergent vegetation and open water, (2) an open-water station located one-half the distance from the emergent station to the wetland center, and (3) a transitional station located one-half the distance between the open water and emergent stations. At each station, four samples were taken using a weighted plant rake, and presence/absence was noted for each species on each of the 60 rake throws in each wetland. The relative abundance measurement for each species consisted of the number of times it was collected on the 60 rake throws. We have previously shown that the commonly observed species of submersed macrophytes in these wetlands occur as three distinct assemblages that exhibit orthogonal gradients of abundance: (1) *Myriophyllum*, (2) *Chara* and *Utricularia vulgaris* (hereafter the "*Chara*" assemblage), and (3) *Ceratophyllum demersum*, *Najas flexilis*, *Stuckenia pectinatus*, *Potamogeton pusillus*, *Potamogeton richardsonii*, and *Potamogeton zosteriformis* (hereafter the "*Potamogetons*" assemblage) (K.D. Zimmer, M.A. Hanson, and M.G. Butler, unpublished data). The plant community in most wetlands was dominated by one of these three groups, and a lesser number of sites had a mixed assemblage of all three groups. Given the distinct gradients of abundance represented by these three groups of macrophytes, we reduced our macrophyte data to three independent variables, *Myriophyllum*, *Chara*, and *Potamogetons*, the value of each assemblage in each wetland consisting of the summed abundance scores of the component species listed above. Use of these three macrophyte variables allowed us to assess whether macrophyte influences on water quality characteristics varied with community composition, without using an unwieldy number of variables.

The abundance of zooplankton was measured on the same dates that we collected water samples, producing six estimates per year in each site. On each date, samples were collected using an integrated column sampler (Swanson 1978) at each of the five open-water stations used to sample macrophytes. Samples were condensed using a 68-μm mesh and were preserved in ethanol. We then determined the number of *Daphnia* and "other cladocerans" (mainly *Ceriodaphnia*, *Simocephalus*, and Chydoridae) in each sample. We focused on cladocerans because of their higher grazing rates relative to copepods (Peters and Downing 1984). Similarly, we distinguished *Daphnia* from other cladocerans because it is thought to be the most effective grazer among the cladocerans (reviewed by Sterner 1989). The number of *Daphnia* and other cladocerans was summed across the five samples in each wetland on each date, and a seasonal mean was calculated from the six sampling dates for each wetland in each year. This seasonal mean was used for all statistical analyses.

Presence/absence and relative abundance of fish was determined for each wetland in late May, mid-July, and mid-August of each year using minnow traps. On each date, five traps were placed in each wetland at the emergent sampling stations and the samples were collected after 24 h. The most commonly captured fish species were fathead minnows, brook sticklebacks (*Cualea inconstans*), and central mudminnows (*Umbra limi*). However, fathead minnows dominated samples in all wetlands with fish and accounted for 98% of the fish biomass (Zimmer et al. 2002). Fish were treated as a categorical variable (present/absent) for all statistical analyses. Our original design consisted of classifying nine wetlands with fish and nine without (based on sampling in spring of 1996), but small numbers of fish were occasionally captured in wetlands classified as fishless in subsequent years. However, abundance of fish in the wetlands classified as "with fish" was over an order of magnitude higher than in wetlands classified as fishless, and none of the fishless wetlands contained fish in consecutive years (Zimmer et al. 2002). Thus, the fishless wetlands with infrequent, low-abundance fish populations were classified as fishless.

We also included drainage history of the wetlands (nondrained or restored) as a categorical, independent variable, because our previous work indicated higher concentrations of P in the restored wetlands compared with nondrained sites (Zimmer et al. 2002).

Statistical analyses

Individual wetlands were our unit of observation and all analyses were performed on seasonal means. Thus, our analyses were based on 90 wetland-year observations (18 wetlands sampled for five years). Our objective was to identify relationships among chlorophyll *a*, P, N, N:P, and turbidity (dependent variables) and assess how these dependent variables related to drainage history, presence/absence of fish, *Myriophyllum*, *Chara*, *Potamogetons*, and the abundance of *Daphnia* and other cladocerans (independent variables). Thus, we used indirect (principal components analysis, PCA) and direct (redundancy analysis, RDA) gradient analyses. We chose these linear ordination methods over unimodal methods because detrended correspondence analysis indicated gradient lengths less than 2 standard deviations (ter Braak 1995). Partial PCA and RDA were used for all analyses to eliminate effects of time (year treated as a covariable), and we used standardized PCA and RDA because of the different scales of our dependant variables (ter Braak and Smilauer 1998). We used both partial PCA and RDA because the former is better at showing relationships among dependent variables and their relationship to individual wetlands, whereas the latter excels at identifying and testing the strength of re-

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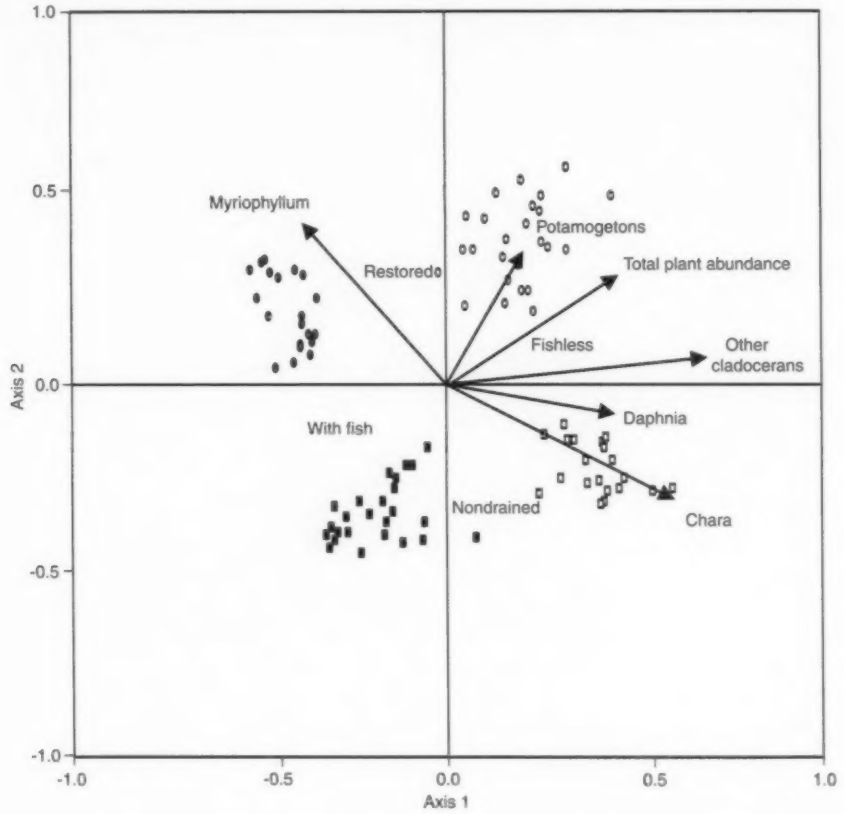
Fig. 1. Correlation biplot produced by standardized partial principal components analysis of eight independent variables hypothesized to influence phytoplankton abundance and nutrient concentrations in the study sites. Vectors for continuous variables are shown as arrows, whereas categorical variables are shown as centroids for each level of presence/absence of minnows and nondrained or restored wetlands. Solid symbols, wetlands with fish; open symbols, wetlands without fish; squares, nondrained wetlands; and circles, restored wetlands.



lationships between dependent and independent variables (ter Braak 1995).

Turbidity was strongly correlated with chlorophyll *a* in our study sites (Zimmer et al. 2002), and we expected that N:P would correlate negatively with P. Given these correlations among the dependent variables, including turbidity and N:P in our analyses would likely inflate the variance explained and perhaps obscure relationships among N, P, and chlorophyll *a*. Thus, turbidity and N:P were treated as supplementary, dependent variables in all analyses. This allows indirect assessment of relationships of N:P and turbidity with the other dependent variables and the independent variables, but as supplementary variables, they do not influence ordination results or contribute to variance explained (ter Braak and Smilauer 1998).

We first used partial PCA to assess relationships among our independent variables. We then used partial PCA to assess relationships among the dependent variables and developed a triplot to indirectly assess relationships among the dependent and independent variables. Similar results with the partial PCA triplot and partial RDA indicate that important independent variables have been identified and that relationships between dependent and independent variables are strong and robust (ter Braak 1994, 1995).

We used partial RDA to explore relationships among dependent and independent variables and to identify independent variables most strongly related to dependent variables. We were not testing a priori hypotheses. Thus, we used forward selection to identify independent variables that explained a significant amount of variance in our dependent variables, and we restricted our partial RDA to independent variables with significant marginal and conditional effects (ter Braak and Verdonschot 1995). Marginal and conditional significance of each independent variable was assessed using permutation tests based on 1000 permutations. We maintained an experimentwise error rate of $P < 0.05$ by applying a sequential Bonferroni correction to both the marginal and conditional effect tests (Rice 1990). Given our use of forward selection and the "exploratory" nature of our analysis, our *P* values should only be considered approximate.

A "full-model" partial RDA was performed using all independent variables with significant marginal and conditional effects, and significance was assessed using a permutation test based on 1000 permutations. We assessed the marginal

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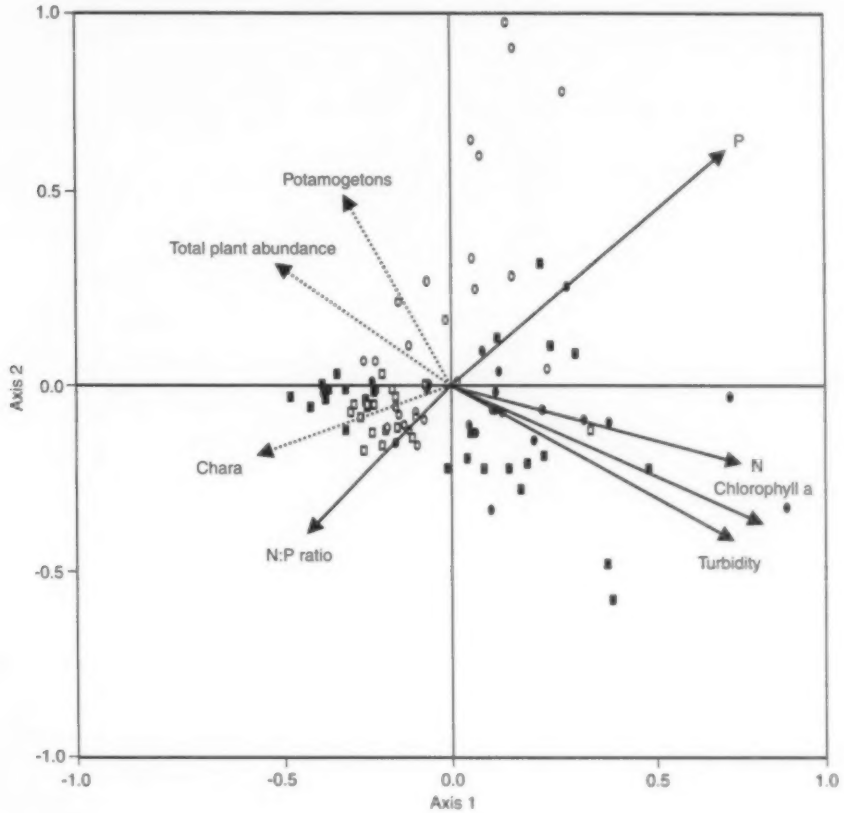
Fig. 2. Correlation triplot produced by standardized partial principal components analysis of the dependent variables chlorophyll *a*, N, and P. Turbidity and N:P were treated as supplementary dependent variables and did not influence the ordination results. Vectors for the dependent variables are shown as solid arrows. Vectors for independent variables were determined post-PCA and are shown as broken arrows. *Chara*, *Potamogetons*, and total plant abundance were the only independent variables with correlations greater than 0.2 for either axis. Solid symbols, wetlands with fish; open symbols, wetlands without fish; squares, nondrained wetlands; and circles, restored wetlands.



effects of each significant independent variable on each dependent variable by performing a series of partial RDAs in which each independent variable was modeled alone and year was treated as the only covariable. We also assessed conditional effects of each significant independent variable using a series of partial RDAs in which each independent variable was modeled alone and all other significant independent variables and year were treated as covariables. In this analysis, marginal effects represent the variance explained by each independent variable (excluding time), some of which may also be explained by other independent variables. In contrast, conditional effects represent explained variance that is "unique" to each independent variable, determined by fitting an independent variable to the residual variation unexplained by all other independent variables.

Finally, we assessed the likelihood of N or P deficiency for phytoplankton growth by plotting seasonal means of N versus P for each wetland, producing a scatterplot of N:P. The N:P for each wetland-year was identified by a circle, with the area of the circle proportional to the seasonal mean value of chlorophyll *a* in that site. We also constructed isopleths for N:P of 7.2, 10, and 20 (by mass). A N:P of 7.2 represents the Redfield ratio, ratios of 10 to 20 indicate moderate P deficiency, and ratios > 20 indicate severe P deficiency (Healy and Hendzel 1980).

Results

Partial PCA of the independent variables indicated negative relationships between the presence of fish and *Potamogetons*, *Chara*, total plant abundance, *Daphnia*, and other cladocerans (Fig. 1). *Myriophyllum* showed stronger, and *Potamogetons* weaker, positive associations with restored wetlands, whereas *Chara* and *Daphnia* showed weak positive relationships with nondrained wetlands. Axes 1 and 2 explained 32 and 25% of the total variance in the independent variables, respectively. The perpendicular relationship between the plant assemblage vectors indicates that the abundances of *Potamogetons* and *Chara* were largely independent, whereas the opposing directions of the *Myriophyllum* and *Chara* vectors indicate a negative relationship between these two variables (Fig. 1). Thus, multicollinearity was weak be-

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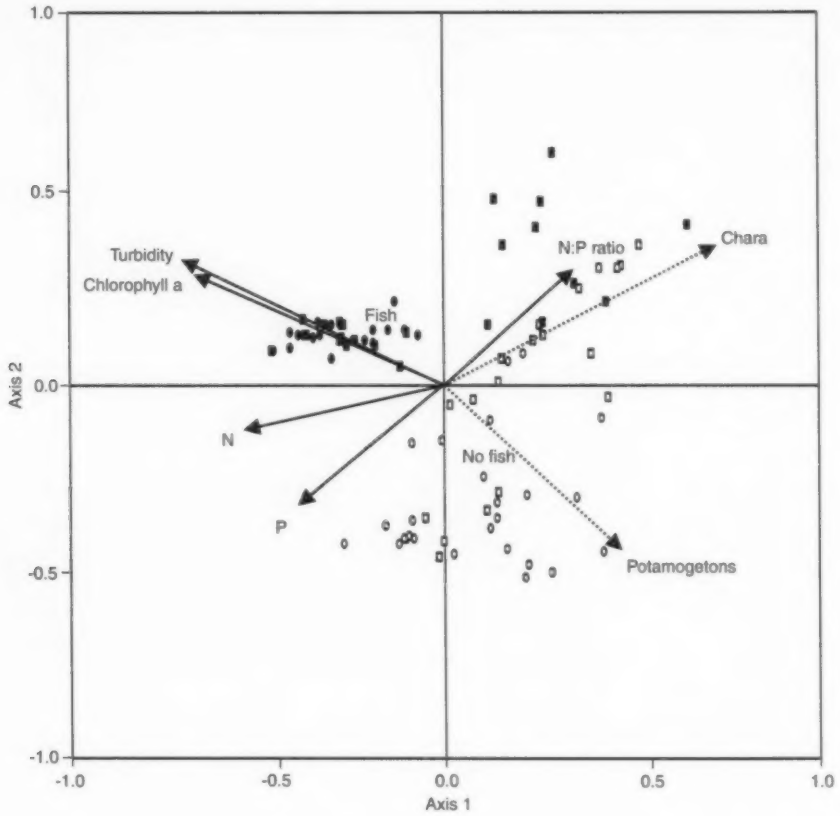
Fig. 3. Correlation triplot produced by standardized partial redundancy analysis of the dependent variables chlorophyll *a*, N, and P and the independent variables presence/absence of fish, *Chara*, and *Potamogetons*. Turbidity and N:P were treated as supplementary dependent variables and did not influence the ordination results. Vectors for the dependent variables are shown as solid arrows, and continuous independent variables as broken arrows. The categorical independent variable presence/absence of fish is shown as the centroid of each level. Solid symbols, wetlands with fish; open symbols, wetlands without fish; squares, nondrained wetlands; and circles, restored wetlands.



tween *Chara* and *Potamogetons*, and the relationships between each of these two macrophyte assemblages and the dependent variables were independent of the other assemblage.

Partial PCA of the dependent variables indicated positive relationships among N, chlorophyll *a*, and turbidity, a negative relationship between P and N:P, and that N:P and P were largely independent of N, turbidity, and chlorophyll *a* (Fig. 2). The individual wetland-years largely segregated based on presence/absence of fish, with fishless wetlands having lower axis 1 and higher axis 2 scores compared with most wetlands with fish. The major exceptions were 10 wetland-years with fish (representing data from two wetlands across all five years) that had scores similar to fishless sites and two fishless wetland-years that scored more similarly to wetlands with fish. P and N:P were more variable in fishless wetlands, whereas N, chlorophyll *a*, and turbidity varied more in wetlands with fish (Fig. 2). Total plant abundance, *Potamogetons*, and *Chara* were the only independent variables with correlations greater than 0.2 for either axis. The abundance of *Chara* and *Potamogetons* again displayed an orthogonal relationship similar to that observed in Fig. 1. *Chara* showed a positive relationship with N:P and negative relationships with all other dependent variables. In contrast, *Potamogetons* showed a negative relationship with N, chlorophyll *a*, and turbidity but was unrelated to P or N:P. Total plant abundance showed relationships intermediate between *Chara* and *Potamogetons*.

Forward selection using partial RDA indicated that *Chara*, fish, total plant abundance, *Potamogetons*, and drainage history were the only independent variables with significant marginal effects, and together they explained 42% of the variance in the dependent variables (Table 2). However, only *Chara*, *Potamogetons*, and fish exhibited significant conditional effects (Table 2). The full-model partial RDA using *Chara*, *Potamogetons*, and fish explained a significant amount of the total variance in the dependent variables (40%, $P = 0.001$), with axes 1 and 2 explaining 29 and 7% of the variance, respectively (Fig. 3). The five independent variables with significant marginal effects explained 42% of the variance in the dependent variables, yet the model using only *Chara*, *Potamogetons*, and fish explained 40% of the vari-

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Table 2. Results of forward selection of independent variables.

Environmental variable	Eigenvalue	*P* value	Cumulative eigenvalue
(*a*) Marginal effects			
Chara	0.178	0.007*	
Total plant abundance	0.159	0.007*	
Fish presence	0.126	0.007*	
Potamogetons	0.103	0.007*	
Drainage history	0.077	0.020*	
Other cladocerans	0.039	0.117	
Daphnia	0.018	0.208	
Myriophyllum	0.009	0.419	
(*b*) Conditional effects			
Chara	0.178	0.004*	0.178
Potamogetons	0.159	0.004*	0.337
Fish presence	0.062	0.004*	0.399
Drainage history	0.020	0.078	0.419
Total plant abundance	0.005	0.388	0.424

Note: Variables with significant marginal effects (*a*) were tested stepwise for significant conditional effects (*b*), with previously entered variables treated as covariables. The overall error rates in both (*a*) and (*b*) were maintained at $P < 0.05$ by applying sequential Bonferroni corrections. * *P* value adjusted with a sequential Bonferroni correction.

Table 3. Percent of total variation in the five dependent variables explained by partial redundancy analysis (RDA) modeling of (*a*) each of the three independent variables individually with year as the only covariable (marginal effects) and (*b*) each of the three independent variables individually with the other two independent variables and year as covariables (conditional effects).

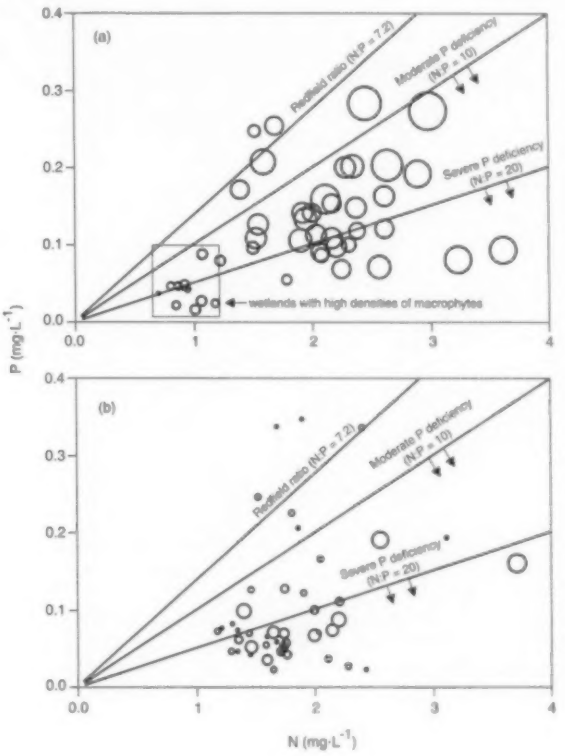
Response variable	*Chara*	*Potamogetons*	Fish presence	Full model
(*a*) Marginal effects				
Chlorophyll *a*	18.9	12.9	36.6	
P	27.1	1.4	0.9	
N	7.4	16.7	0.4	
N:P	33.5	4.8	3.6	
Turbidity	21.9	13.4	45.0	
Total variance	17.8	10.3	12.6	
(*b*) Conditional effects				
Chlorophyll *a*	9.3	12.8	10.6	52.9
P	27.0	0.4	2.6	29.7
N	20.5	29.8	5.5	37.2
N:P	24.4	0.0	0.0	34.4
Turbidity	9.5	12.6	14.4	61.2
Total variance	18.9	14.3	6.2	39.9

Note: Conditional effects also show the "full" model RDA, in which all three independent variables were used simultaneously in one analysis and year was the only covariable. All analyses were conducted on chlorophyll *a*, P, and N; N:P and turbidity were supplementary variables. Total variance represents the average variance explained across chlorophyll *a*, P, and N.

ance. This is further evidence that these latter three independent variables were most strongly related to our dependent variables.

Relationships among the dependent variables shown in Fig. 3 were very similar to those indicated in Fig. 2, although the associations between N and chlorophyll *a* and between N and turbidity were weaker than in Fig. 2. Vectors

Fig. 4. Relationships among concentrations of N (*x* axis), P (*y* axis), and chlorophyll *a* (size of circle) in (*a*) wetlands with fish and (*b*) fishless wetlands. Values represent seasonal means of the 18 wetlands in five different years. Isopleths show N:P (by mass) indicating severe (>20) and moderate (10–20) P deficiency (based on Healy and Hendzel 1980), as well as the Redfield ratio of 7.2.



for *Chara* and *Potamogetons* were again orthogonal, and *Chara* displayed a positive relationship with N:P and negative relationships with all other dependent variables. In contrast, *Potamogetons* showed a strong negative relationship with chlorophyll *a* and turbidity, a weaker negative association with N, and no apparent relationship to P or N:P. Nearly all wetland-years with fish had much higher levels of turbidity and chlorophyll *a* than did fishless wetlands. In contrast, N, P, and N:P were similar in wetlands with and without fish, although P and N:P varied more among the fishless wetlands. Most wetlands with fish exhibited little variance and had negative scores for axis 1 and positive scores for axis 2 (Fig. 3). The exceptions were 10 wetland-years with fish (representing two wetlands across all five years) that had positive scores for axes 1 and 2. These sites had chlorophyll *a* and turbidity values intermediate between fishless sites and all other sites with fish and much lower values of N and P and higher N:P compared with other wetlands with fish. As indicated by the *Chara* vector, these two sites had much higher abundance of *Chara* compared with all other wetlands with fish.

Analysis of marginal and conditional effects indicated that the variance explained in each of the five dependent vari-

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ables differed among the independent variables (Table 3). Marginal effects showed that *Chara* and *Potamogetons* explained the most and the least variance in the dependent variables, respectively. For marginal effects, *Chara* explained the most variance in P and N:P, *Potamogetons*, the most variance in N, and fish, the most variance in chlorophyll *a* and turbidity. Conditional effects indicated that *Chara* and fish explained the most and the least variance, respectively, a result of the explanatory power of fish for chlorophyll *a* and turbidity dropping considerably after the effects of *Chara* and *Potamogetons* were removed. In contrast, variance in P and N:P explained by *Chara* decreased little from the marginal effects, indicating that this source of explained variance is independent of all other independent variables. A surprising result was that variance in N explained by both *Chara* and *Potamogetons* was higher in the conditional effects than in the marginal effects. This indicates that the ability of *Chara* and *Potamogetons* to explain N variance is strongly dependant on one another. Using all three independent variables in the full-model partial RDA, variance explained was highest for chlorophyll *a* and turbidity, moderate for N, and lowest for P and N:P.

The N:P of most wetland-years (with and without fish) indicated either moderate or severe P deficiency for phytoplankton growth (Fig. 4). In wetlands with fish, N:P of 5 wetland-years indicated no P deficiency (N:P < 10), of 23, moderate P deficiency (N:P 10 to 20), and of 17, severe deficiency (N:P > 20). In fishless wetlands, N:P of 6 wetland-years indicated no deficiency, of 11, moderate deficiency, and of 28, strong deficiency. The 10 wetland-years with fish that exhibited much higher abundance of *Chara* compared with all other wetlands with fish (see Fig. 3) had distinctly lower mean values of N and P compared with all other wetlands with fish.

Discussion

Our results indicated that abundance of submersed macrophytes and presence/absence of minnows have strong influences on phytoplankton biomass and nutrient concentrations in prairie wetlands. However, *Chara* and *Potamogetons* explained three- and two-fold more variance, respectively, than did presence/absence of fish. Additionally, relationships with nutrients differed between these two groups of plants, as *Chara* showed strong negative relationships with both N and P, whereas *Potamogetons* showed a strong relationship with N only. Furthermore, variance in P and N:P was greater in fishless wetlands compared with wetlands with fish, likely driven by the more variable abundance of *Chara* among fishless wetlands and its strong negative effect on P. Overall, these results indicated that submersed macrophytes have strong influences on water-quality characteristics of prairie wetlands and that nutrient dynamics may vary among wetlands with differing assemblages of submersed macrophytes.

As noted earlier, fishless wetlands used in this study had higher abundance of nearly all submersed macrophytes and lower phytoplankton biomass compared with wetlands with fish (Zimmer et al. 2002; K.D. Zimmer et al., unpublished data). Thus, it may seem that forward selection of *Chara*, *Potamogetons*, and fish resulted from multicollinearity among these three independent variables. However, our analysis of conditional effects showed that each variable explained a unique variance component, and variance explained in the dependent variables varied among independent variables. *Chara* and *Potamogetons* best explained variance in N, P, and N:P, whereas variance in phytoplankton abundance and turbidity was explained similarly by all three independent variables. Additionally, the "full" model RDA explained two- to four-fold more variance than did models using any one of our independent variables. Although some variance explained in the dependent variables was shared among our independent variables (particularly for chlorophyll *a* and turbidity), use of all three independent variables produced a much clearer picture of factors influencing nutrient concentrations and phytoplankton abundance in these ecosystems.

Numerous studies have documented influences of submersed macrophytes on phytoplankton abundance and nutrient concentrations in shallow waters (Hanson and Butler 1994; Jeppesen et al. 1999; Blindow et al. 2000), but we are unaware of studies documenting site-level differential effects based on community composition in prairie wetlands or other ecosystems. The negative relationship of N with both *Chara* and *Potamogetons* is likely driven by increased uptake of water-column N by epiphytic algae and perhaps by macrophytes creating oxic–anoxic gradients that promote denitrification (reviewed by van Donk and van de Bund 2002). For phosphorus, strong influences of *Chara* on P concentrations and availability have been observed previously (Kufel and Ozimek 1994; Blindow et al. 2000), and influences of *Chara* on N:P observed here probably reflect relationships between *Chara* and P. A surprising result was the absence of a relationship between *Potamogetons* and P, as higher abundance of submersed macrophytes in general is often associated with reduced abundance of P (reviewed by Scheffer 1998). Why a similar relationship was not evident for the *Potamogeton* assemblage in our study is unclear, but it may indicate that nutrient dynamics differ between wetlands dominated by *Chara* and wetlands dominated by other submersed species. Although N:P in most sites indicated moderate to severe P deficiency for phytoplankton growth, N deficiency seems most likely in wetlands dominated by taxa other than *Chara*. In contrast, P limitation may be more widespread in *Chara*-dominated systems. However, N:P are only rough indicators of nutrient deficiency (Healey and Hendzel 1980); nutrient limitation in eutrophic systems often occurs over short time scales, contributing variance not evident in the seasonal means used here (Barcia 1990). Nonetheless, our results may indicate that phytoplankton growth is more constrained by P than by N, at least in *Chara*-dominated wetlands.

Using a factorial approach on data from the same wetlands, we previously reported that P concentrations were higher (Zimmer et al. 2002) and *Chara* abundance was lower (K.D. Zimmer, M.A. Hanson, and M.G. Butler, unpublished data) in restored wetlands relative to similar nondrained sites. Results presented here suggested that higher P in restored sites may reflect lower abundance of *Chara* compared with nondrained analogs, rather than more obvious effects associated with prior drainage. This hypothesis is supported by results of variance partitioning using *Chara* and drainage history. A partial RDA using *Chara* and drainage history as independent variables (year as covariable) indicated that

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these two variables explained 35% of the total variance in P (data not shown). Partial RDAs indicated that "pure" *Chara* explained 14% (drainage history and year as covariables), pure drainage history explained 7% (*Chara* and year as covariables), and 14% of the variance was shared.

It is plausible that alternative ecosystem characteristics both induced the differences in macrophyte community composition and, simultaneously, produced the observed differences in P concentrations among our study sites. Particle size and percent organic matter of sediments, as well as water-column conductivity, alkalinity, and pH, influenced submersed macrophyte communities (Chambers and Prepas 1990; Vestergaard and Sand-Jensen 2000; Lougheed et al. 2001). These and other factors may play important roles in determining relative abundance of *Chara* and *Potamogetons* in our study sites (K.D. Zimmer, M.A. Hanson, and M.G. Butler, unpublished data). Whether these factors caused observed differences in P concentrations among our study wetlands is unknown. Further work is clearly needed to decipher whether the differential effects of *Chara* and *Potamogetons* on P are in fact due to the plants or result from other ecosystem characteristics that covary with community composition of submersed macrophytes.

We observed no strong relationships among abundance of *Daphnia* (or other cladocerans), phytoplankton, and nutrient concentrations. This was a surprising result, as several studies have documented strong influences of cladocerans in shallow, eutrophic waters, particularly on phytoplankton abundance (Hann and Goldsborough 1997; Stephen et al. 1998; Jeppesen et al. 1999). However, our inability to detect strong relationships may be due to our use of seasonal means for the cladoceran data. Cladoceran abundance often exhibits high temporal variability during the growing season, with a general trend toward high abundance early in the growing season and declining populations as the season progresses (Jeppesen et al. 1997; Stansfield et al. 1997). Early-season peaks in abundance may be especially important for inducing "clear-water" phases that facilitate subsequent, annual growth of submersed macrophytes (Hanson and Butler 1994). Thus, cladocerans probably have strong influences on phytoplankton over shorter time scales and also indirectly influence phytoplankton abundance and nutrient availability by facilitating growth of submersed macrophytes. However, the consequences of relatively short periods of high zooplankton abundance and grazing rates are probably not reflected in our data because of our use of seasonal mean abundance.

The observed strong marginal effects of fish on phytoplankton are consistent with the notion that cladoceran abundance had an important influence on algal biomass. Previous work in these sites (Zimmer et al. 2002) and other prairie wetlands (Hanson and Riggs 1995) has shown that presence of minnows is associated with sharply reduced abundance of large-bodied cladocerans. Fish influence abundance of phytoplankton in two major ways: (1) by directly or indirectly modifying nutrient availability or altering nutrient ratios, and (2) by reducing zooplankton abundance and grazing pressure (Vanni and Layne 1997). Analysis of marginal effects indicated that fish had strong positive associations with chlorophyll *a* and turbidity, but they showed virtually no relationship with N, P, or N:P. This indicates that direct influences of fish on nutrient dynamics and availability were weak (mechanism 1 above), thus fish-generated differences in cladoceran abundance were likely responsible for observed relationships between fish and phytoplankton.

Relationships among phytoplankton abundance, nutrient concentrations, presence of fish, abundance of zooplankton, and abundance of macrophytes are consistent with predictions of alternative stable-state models proposed by Scheffer et al. (1993) and others. All our fishless wetlands had more extensive development of submersed macrophytes and much lower phytoplankton biomass compared with wetlands with fish. Thus, it appears that wetlands with fish exhibited characteristics of a turbid-water state, whereas fishless wetlands exemplified the clear-water alternative. Exceptions to this pattern were two study wetlands with fish that developed moderate phytoplankton concentrations, low nutrient concentrations, and much higher abundance of macrophytes compared with other wetlands with fish. As abundance of zooplankton was sharply reduced in all of the wetlands with fish (Zimmer et al. 2002), these patterns indicated that the high abundance of macrophytes in these two sites maintained lower phytoplankton abundance by reducing nutrient availability (Blindow et al. 2000). Our data provided evidence of a strong influence of submersed macrophytes, especially *Chara*, in prairie wetlands, and indicated that macrophytes present a strong buffer against shifts to the turbid-water state (sensu Scheffer 1998).

Mechanisms regulating phytoplankton biomass and nutrient concentrations in shallow-water ecosystems are complex, but our results suggest that presence/absence of fish and abundance and community composition of submersed macrophytes are particularly important in prairie wetlands. Similar to other shallow-water ecosystems, an important area of research is identifying mechanisms that induce a reduction in the abundance of submersed macrophytes, facilitating a shift from the clear- to turbid-water state.

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Resistance, resilience, and patchiness of invertebrate assemblages in native tussock and pasture streams in New Zealand after a hydrological disturbance

Adriano S. Melo, Dev K. Niyogi, Christoph D. Matthaei, and Colin R. Townsend

Abstract: We generated hydrological disturbances to investigate the role of disturbance in New Zealand streams in two land uses: native tussock grasslands and exotic pasture catchments. We tested whether physical differences in streambed structure confer higher resistance and resilience in tussock sites than in pasture sites. We also investigated changes in patchiness (at spatial scales larger than 0.06 m^2) caused by the disturbance. Invertebrate abundance decreased immediately after the disturbance. Species density remained unchanged, but species richness (rarefied) increased. Eight days after the disturbance event, abundance and species richness (rarefied) were similar to those of samples collected immediately before the disturbance. Resistance (measured as decrease in abundance) and resilience (measured as recovery within 8 days) did not differ significantly between the land uses. Patchiness increased in both stream types immediately after the disturbance but decreased to predisturbance levels after 8 days. Disturbance caused a redistribution of individuals among patches, some receiving individuals, others losing individuals, and some remaining unchanged. Our results conform with predictions of the patch dynamics concept and are consistent with results of studies of natural disturbance caused by floods.

Résumé : Nous avons produit expérimentalement des perturbations hydrologiques afin d'étudier le rôle de ces perturbations dans des cours d'eau de Nouvelle-Zélande dans des deux régions d'utilisation différente des terres, des bassins versants de prairies de tussack indigènes et de pâturages de plantes exotiques. Nous avons vérifié si des différences physiques dans la structure du lit des cours d'eau des prairies leur confèrent une résistance et une résilience plus grandes que les cours d'eau des pâturages. Nous avons aussi étudié les changements dans la structure en parcelles (aux échelles spatiales supérieures à 0,06 m^2) dus aux perturbations. L'abondance des invertébrés diminue immédiatement après la perturbation. La densité des espèces reste la même, mais la richesse en espèces (calculée par raréfaction) augmente. Huit jours après l'épisode de perturbation, l'abondance et la richesse en espèces (par raréfaction) sont semblables à ce qu'elles étaient dans les échantillons récoltés immédiatement avant la perturbation. La résistance (mesurée par la diminution de l'abondance) et la résilience (mesurée par la récupération au bout de 8 jours) ne diffèrent pas dans les deux bassins dont l'utilisation des terres est différente. La structure en parcelles augmente dans les deux types de cours d'eau immédiatement après la perturbation, mais elle retourne aux niveaux observés avant la perturbation après 8 jours. La perturbation cause une redistribution des individus parmi les parcelles, certaines perdant des individus, d'autres en acquérant, d'autres enfin restant inchangées. Nos résultats sont en accord avec les prédictions du concept de la dynamique des parcelles et sont compatibles avec les résultats d'études des perturbations naturelles causées par les crues.

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A.S. Melo.[1,2] Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand, and Curso de Pós-Graduação em Ecologia, Museu de História Natural, Instituto de Biologia, Universidade Estadual de Campinas, Campinas 13083-970, SP, Brazil. **D.K. Niyogi,**[3] **C.D. Matthaei, and C.R. Townsend.** Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand.

[1]Corresponding author (e-mail: grumicha@yahoo.com.br).
[2]Present address: Departamento de Biologia Geral, ICB1, Universidade Federal de Goiás, CP 131, Goiânia 74001-970, GO, Brazil.
[3]Present address: Department of Biological Sciences, University of Missouri–Rolla, Rolla, MO 65409, U.S.A.

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Introduction

Natural physical disturbance has been recognized by ecologists as an important factor determining the structure of communities (Sousa 1984; Resh et al. 1988). In streams, high discharge events are thought to be the main disturbance structuring invertebrate communities (Townsend 1989; Lake 2000), reducing abundances of most species (Scrimgeour et al. 1988; Hax and Golladay 1998), and redistributing individuals among habitats (Palmer et al. 1996). Recovery following a spate or flood is usually fast, and original abundances are generally regained within 10–120 days depending on disturbance intensity (Mackay 1992; Flecker and Feifarek 1994).

High discharge events can affect stream invertebrates directly in two ways: by detachment from the substrate or as a result of movement of stones with the potential to crush or bury individuals. The first mechanism is indicated by the dislodgement of increasing numbers of invertebrates as discharge in experimental channels is increased (Holomuzki and Biggs 1999) and by the concentration in hydraulic refuges, after floods, of species that are vulnerable to flow detachment (Lancaster 2000). The second mechanism is indicated by higher postflood densities on embedded stones than on loose stones (Matthaei et al. 2000), weak effects of floods in stream reaches with high sediment stability (Cobb et al. 1992), and high mortality in experimental channels containing large, mobile substrate particles (Holomuzki and Biggs 1999). Additionally, disturbance can differentially affect patches that differ in physical structure and stability (Downes et al. 1997), for example, sites with high physical heterogeneity offer a range of hydraulic refuges (dead zones).

Natural physical disturbance is known to induce patchiness in the distribution of organisms in terrestrial (Segura et al. 1998) and marine systems (Underwood 1998), but few studies have specifically addressed this issue in stream systems (Lake 2000). Lancaster and Hildrew (1993) showed that some insect species respond to floods by concentrating in patches where shear stress does not increase with an increase in discharge, whereas Palmer et al. (1995) observed that larval chironomids and copepods were redistributed among habitats following a disturbance. The patch dynamics concept of stream communities (Townsend 1989) suggests that disturbances cause a mosaic of patches, some losing individuals, some not affected, and some receiving individuals. Fast recolonization of depleted patches is possible due to the existence of nearby refuges. Given a long recurrence interval, communities would be expected to have greatest patchiness immediately following a disturbance and then tend toward a more even distribution of individuals among patches until a new disturbance resets the system. In the context of this study, patchiness is defined as aggregated distribution in which each unit (patch) is larger than 0.062 m^2 (the area included by our sampling device) and spaced at least 10 m from each other (the minimum distance between the sampled areas on each sampling occasion).

The study of physical disturbance in streams can be assisted by the use of manipulative experiments to simulate discharge disturbance (Matthaei et al. 1996). In contrast to previous studies, which were generally concerned with small-scale impacts (single stones up to 10 m^2) and located in a single stream (e.g., Douglas and Lake 1994; Matthaei et al. 1996), we applied a hydrological disturbance to reaches 60–90 m long in replicated stream sites. We examined resistance, resilience, and patchiness of macroinvertebrate assemblages following disturbance in streams draining exotic pasture or native tussock grassland. We hypothesized that tussock streams may have greater resistance and resilience than pasture streams because tussock sites are more heterogeneous, which could provide a greater range of hydraulic refuges.

Materials and methods

Study area

The study was carried out in second- and third-order tributaries of the Taieri and Waipori rivers (45°43′S, 169°51′E, total area encompassing all sites = 132 km^2) in the Otago Province of the South Island of New Zealand. Catchment areas ranged from 0.74 to 1.43 km^2 with the exception of Broad Stream (Table 1). Broad Stream had a larger catchment area, but much of the water upstream has been diverted from the catchment to a reservoir so the stream was comparable in size to the others. All study streams contained brown trout (*Salmo trutta* L.), an introduced species that can affect invertebrate behaviour and community composition (Townsend 1996).

The streambed at all sites was composed mainly of cobbles and gravels. Tussock streams had lower mean particle sizes than pasture streams because they had more small particles, sometimes beneath moss (Table 1). Streams in tussock catchments were partially shaded by overhanging grasses, especially species of *Chionochloa* and *Festuca*. Grazing by sheep occurred in tussock catchments historically, but only at low intensity and only during some periods of the year. Grazing had not occurred in our tussock catchments for at least 2 years. Streams in pasture catchments were unshaded, and grazing by sheep and cattle was intense but varied among streams. It is possible that such variation contributed to between-stream variation, but we were unable to address this question.

A large rainfall event occurred in our study area in September 2000, more than 5 months before our experimental disturbances. Our disturbances and sampling occurred during a prolonged dry period in late February and early March of 2001. The only substantial rainfall (12.5 mm) in this period, recorded at a nearby gauge 1 week before our experiment (P. Stevenson, Otago Regional Council, Private Bag 1954, Dunedin, 9001, New Zealand, unpublished data) did not cause increased flow and could not have affected invertebrate communities in our streams. During our study, we observed no sign in the riparian vegetation of a recent flood (such as flattened vegetation or dead leaves trapped in the vegetation). There was no significant rainfall while the experiment was being performed.

Physicochemical properties

Physical and chemical characteristics of the streams were determined following standard methods. Reach gradient was determined with a level and a stadia rod. Width, depth, velocity, and moss cover were determined at 10 cross-sections

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Table 1. Physicochemical characteristics of stream sites.

	Tussock			Pasture		
	Anchor	Clarks	Stone	Broad	Bush	M. Road
Drainage area (km^2)	1.21	1.06	0.74	6.04	1.32	1.43
Elevation (m)	540	600	540	480	580	450
Reach gradient (%)	2.6	3.9	4.9	2.5	4.9	2.5
Velocity ($m \cdot s^{-1}$)	0.11	0.13	0.15	0.11	0.10	0.20
Width (m)	0.66	0.66	0.79	0.71	1.40	1.21
Depth (m)	0.32	0.22	0.22	0.28	0.20	0.13
Moss cover (%)	58	64	52	9	0	0
DRP ($\mu g \cdot L^{-1}$)	2.5	1.8	0.9	13.7	6.1	4.9
DIN ($\mu g \cdot L^{-1}$)	1.6	2.5	2.1	61.2	15.6	5.6
MPS (mm)	25	26	11	60	92	10
CV-PS (%)	128	120	161	106	90	73

Note: DRP, dissolved reactive phosphorus; DIN, dissolved inorganic nitrogen; MPS, median particle size of streambed substrates; CV-PS, the coefficient of variation for particle size. Values for width, depth, velocity, and moss cover are means obtained from measures at 10 cross-sections along each stream.

along each stream. Velocity was measured using a flowmeter (Marsh-McBirney Flo-Mate Model 2000; Marsh-McBirney Inc., Frederick, Md.). A Wolman pebble count of at least 100 particles was conducted to determine the median particle size of streambed substrates (Wolman 1954). Concentrations of dissolved reactive phosphorus (DRP) and dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_3^- + NO_2^-$) were measured (two replicates on each of three visits to the sites) by colorimetric methods on an autoanalyzer.

Experimental design and the disturbance event

The experiments were done using a repeated-measures design. Samples collected immediately before the experimental disturbance were considered controls, whereas samples collected 2 h and 8 days after the disturbance were used to measure resistance and resilience of the invertebrate community, respectively. Three replicates of each stream type were used, totaling 18 samples. Each sample comprised four sampling units, collected at depths of 10–40 cm in similar riffle areas using a Surber sampler (area = 0.062 m^2).

An experimental hydrological disturbance was applied to the streams by means of a water compressor and hose to simulate the high velocities and shear stresses that occur during a flood. In each stream, we systematically hosed a 60- to 90-m reach for about 1 h. The operator moved downstream, "washing" the streambed and taking care to apply the water jet to all portions of the bed, including riffles, pools, and areas under overhanging vegetation and banks. The experimental disturbance was enough to turn the water completely turbid, move stones, and cause the detachment of portions of moss in tussock sites. As a measure of disturbance intensity, shear stress caused by the water jet was sufficient to move even the heaviest standard hemisphere developed by Statzner and Müller (1989) to measure shear stress.

Sampling units collected using the Surber sampler were preserved individually in the field. In the laboratory, we split each sampling unit into two halves and sorted all individuals from one-half (because of the large number of individuals). Invertebrates were identified to the lowest taxonomic level possible. In cases in which species identification was not possible, we separated individuals into morphospecies.

Analysis

Resistance and resilience in pasture vs. tussock streams

Resistance and resilience of the invertebrate assemblages were assessed using a nested two-way repeated-measures analysis of variance (ANOVA), with land use (pasture, tussock) as the between-subjects factor and time (before, after, and 8 days after) as the within-subject factor. Data from sampling units (Surbers) were nested within streams, the subject of the analysis. Because of the nonindependent levels of the time factor and the consequent potential problems in the variance–covariance matrix, p values were corrected for time and interaction factors by the Huynh–Feldt method (Gurevitch and Chester 1986; Looney and Stanley 1989). We set two a priori comparisons in case the within-subjects factor (time) was significant: before vs. after and before vs. 8 days after sampling times. Analyses were carried out using NCSS software (Hintze 2001). Because of the low replication of streams possible in our large-scale experiment, the power of our analysis was generally relatively low and we set the significance level for α (alpha) at 0.10.

Three metrics were used in the ANOVA analysis: (1) $\log_{10}$ abundance, (2) species density (number of species in sampling units), and (3) species richness (expected values derived by rarefaction-like resampling; see Gotelli and Colwell 2001). Values of species richness were obtained using EcoSim software (Gotelli and Entsminger 2001). The lowest abundance value found in a Surber sampling unit over the three time periods was used in the rarefaction of the other 17 sampling units. The reason for including rarefaction estimates of species richness was the findings of McCabe and Gotelli (2000) that a decrease in species density in a small-scale disturbance experiment was a consequence of reduced abundance in disturbed treatments.

Although we have assessed statistically the differences among catchment types using the repeated-measures ANOVA analysis described above, we illustrate results of our experiment regarding abundance using indexes of resistance and resilience. Resistance was obtained as the total abundance (sum of four sampling units) found immediately after the disturbance divided by the total abundance before the distur-

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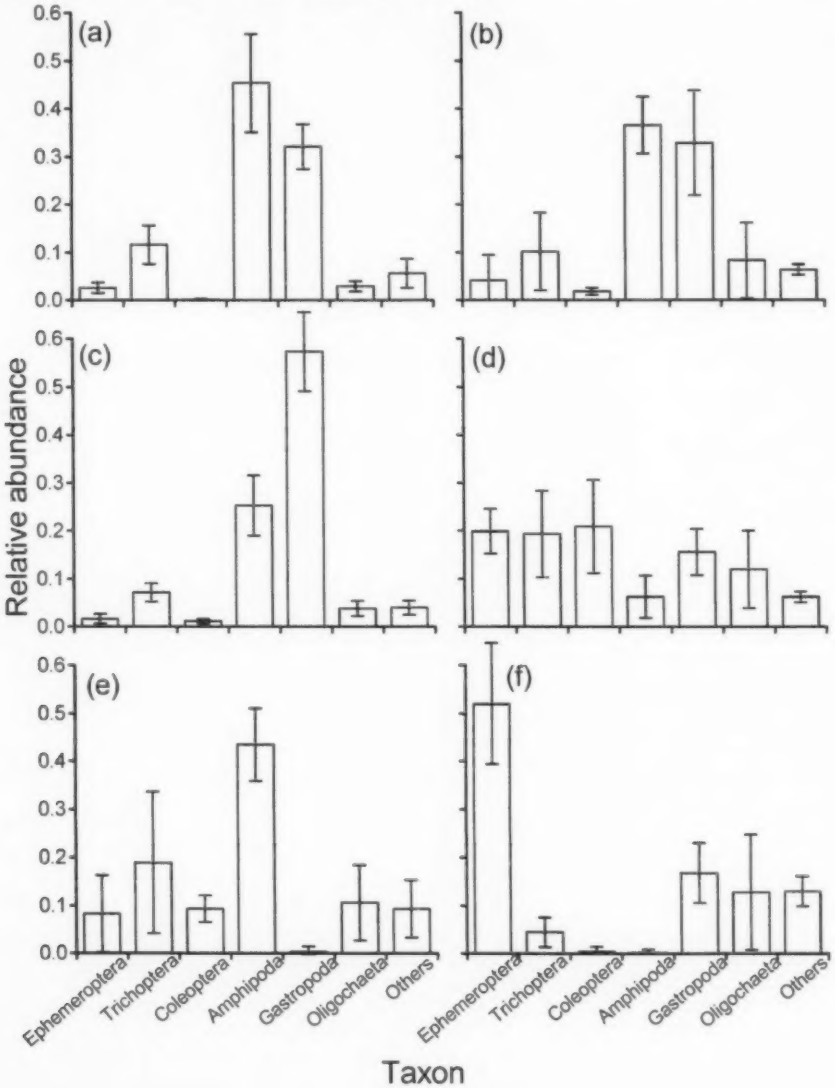
Fig. 1. Main invertebrate groups found in the six streams studied (left column, tussock streams; right column, pasture streams): (*a*) Anchor; (*b*) Broad, (*c*) Clarks, (*d*) Bush, (*e*) Stone, and (*f*) M. Road. Most individuals of Ephemeroptera in Bush and M. Road belonged to *Deleatidium* spp. (Leptophlebiidae). Nearly all individuals of Gastropoda and Amphipoda collected were of the genera *Potamopyrgus* and *Paracalliope*, respectively. Data are from samples collected immediately before the disturbance event. Error bars indicate standard deviation calculated using four Surber sampling units.



bance event. Similarly, we computed resilience as the total abundance found 8 days after the disturbance divided by the total abundance before the disturbance event. Our original predictions were that tussock sites would have higher resistance and resilience than pasture sites, reflecting the availability of more refuges in the former.

Patchiness following the disturbance

We investigated changes in patchiness within a stream site in an exploratory way using a nonmetric multidimensional scaling (NMS) ordination of the four sampling units collected in each stream site and sampling time. We used Sørensen distances for $\log_{10}$-transformed abundances and performed the ordination in two dimensions. The analysis was carried out in PC-ORD software (version 4.10, MjM Software, Gleneden Beach, Oreg.) using all 72 sampling units (2 stream types × 3 replicated stream sites × 3 sampling times × 4 sampling units). However, we plotted results separately for each stream site because of the large number of sampling units. We expected that sampling units collected in the same site before the disturbance would be positioned close to each other in ordination space, reflecting high simi-

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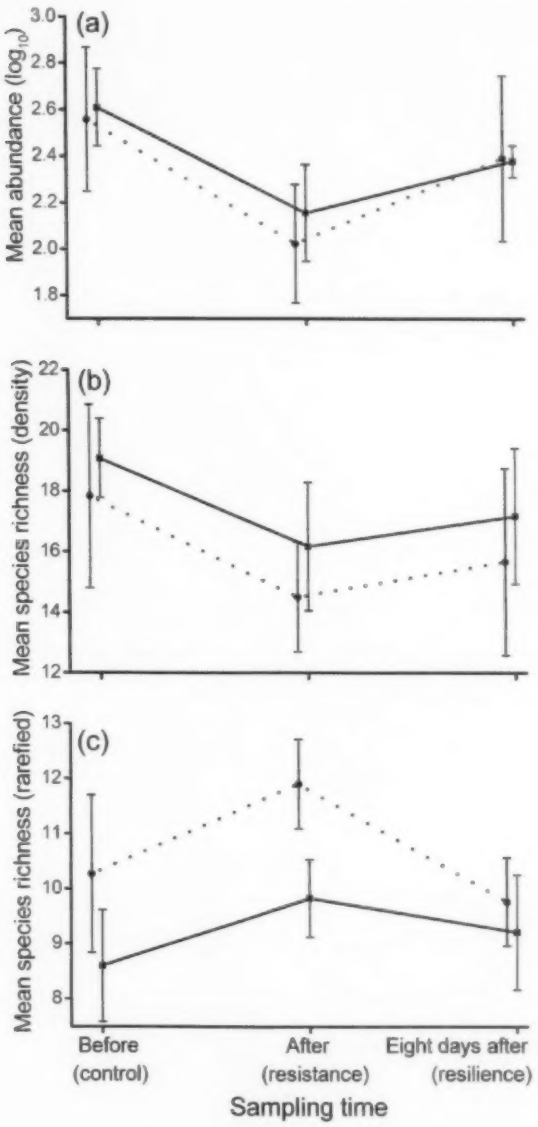
Fig. 2. Mean invertebrate (*a*) abundance, (*b*) species density, and (*c*) species richness (rarefied) in tussock (solid lines) and in pasture (dotted lines) stream sites for three sampling times. Standard errors are shown; $n = 3$ for each sampling time and catchment type.



larity in assemblage composition and abundance. On the other hand, we expected that sampling units collected after the disturbance would be scored far from each other, reflecting low similarity among assemblages.

We also tested the hypothesis of increased patchiness (or heterogeneity) following the disturbance, and a subsequent decline, using a similar ANOVA design to that used in the analysis of resistance and resilience but without the nesting factor. The metric that we used was the mean of the squared

Table 2. Repeated-measures analysis of variance (ANOVA) of effects of land use (tussock vs. pasture) on (*a*) $\log_{10}$ abundance, (*b*) species density, and (*c*) species richness (rarefied).

	df	MS	*F*	*p*
(*a*) $\log_{10}$ abundance				
Between subjects				
LU	1	0.057	0.029	0.873
Streams(LU)	4	1.994		
Within subjects				
T	2	1.482	16.124	0.003
T × LU	2	0.031	0.339	0.693
T × Stream(LU)	8	0.092		
Residual	54	0.101		
(*b*) Species density				
Between subjects				
LU	1	39.014	0.265	0.634
Streams(LU)	4	147.306		
Within subjects				
T	2	60.43	2.334	0.159
T × LU	2	0.264	0.010	0.990
T × Stream(LU)	8	25.889		
Residual	54	10.134		
(*c*) Species richness (rarefied)				
Between subjects				
LU	1	36.871	1.250	0.326
Streams(LU)	4	29.487		
Within subjects				
T	2	15.745	5.012	0.039
T × LU	2	3.712	1.182	0.355
T × Stream(LU)	8	3.141		
Residual	54	2.514		

Note: *T*, time; LU, land use. Error term for the within-subject *F* tests was the interaction *T* × Stream(LU). *p*-values for within-subjects analysis were corrected using Huynh-Feldt method for repeated-measures ANOVA. Symbols "×" and "( )" indicate, respectively, crossing and nesting relationships among variables.

Sørensen similarities (1 – Sørensen distance) from all six possible pairwise comparisons between the four sampling units collected at each stream site and sampling time. We did not nest the six similarity values within streams because each sampling unit would then be used more than once in the calculations, thus failing to meet the assumption of independence. Sørensen similarities were calculated using $\log_{10}$-transformed abundance. Our prediction was that similarity among sampling units would be high before the disturbance (low patchiness), decrease immediately after, and then increase toward original levels after 8 days.

Results

Physicochemical characteristics

Stream width, depth, velocity, and slope varied across sites within tussock and pasture land uses (Table 1). However, the range of values in the two land uses showed considerable overlap. Tussock streams had greater substrate heterogeneity, as indicated by a higher coefficient of variation for substrate particle size. Mosses were abundant at tussock sites, covering

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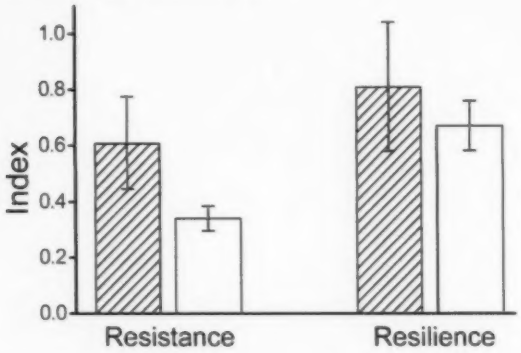
Fig. 3. Average resistance and resilience of the macroinvertebrate assemblage in the face of a discharge disturbance in tussock (hatched bars) and pasture streams (open bars). Resistance was obtained as abundance immediately after the disturbance divided by abundance immediately before it. Resilience was obtained as abundance 8 days after the disturbance divided by abundance immediately before the disturbance. Standard errors are shown; n = 3 streams for each catchment type.



about half of the bed. Moss was present at only one pasture site, covering 9% of the bed. As would be expected, concentrations of dissolved reactive phosphorus and dissolved inorganic nitrogen were higher in pasture than in tussock streams.

Invertebrate assemblages

Invertebrate assemblages differed between streams in pasture and tussock catchments (Fig. 1). The most conspicuous difference was the high proportional abundance of mayflies (mostly *Deleatidium* spp.) in two of the three pasture sites. Invertebrate assemblages in streams in tussock catchments were dominated by *Potamopyrgus* snails and *Paracalliope* amphipods. One pasture site (Broad) had abundant snails and amphipods as well.

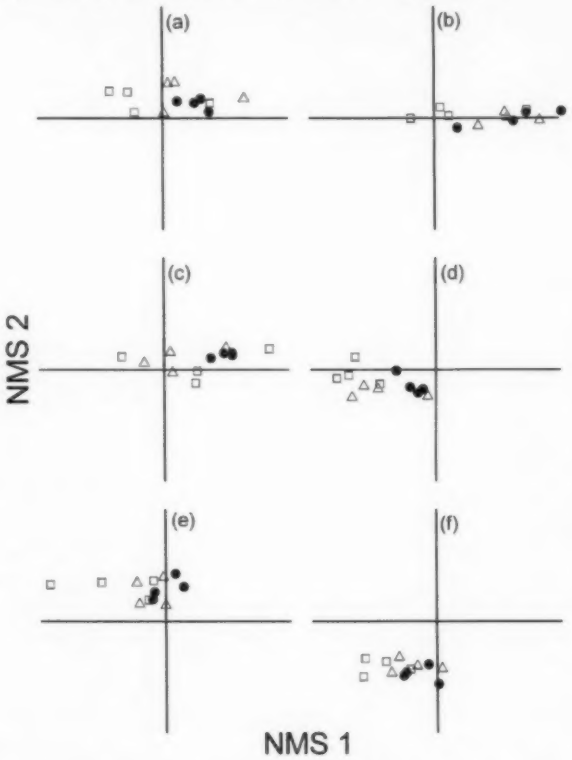
Resistance and resilience in tussock vs. pasture streams

Abundance decreased significantly in streams from both land uses immediately after the experimental disturbance (Fig. 2*a*; Table 2; a priori comparison before vs. after, $p < 0.001$). By 8 days after the disturbance, abundance of invertebrates had recovered to levels quite close to those of control samples (Fig. 2*a*; Table 2; a priori comparison before vs. 8 days after, $p = 0.053$). Resistance tended to be higher in the tussock streams, whereas resilience was similar for tussock and pasture streams (Fig. 3). However, overall there was no significant interaction between land use and time ($p = 0.693$; Table 2), indicating that the two stream types responded in a statistically indistinguishable manner to the disturbance.

There was no clear, consistent pattern in the response of species density by land use types. The effects of land use, time, and the interaction between them were not significant (for all cases, $p > 0.15$; Table 2). Species density did not change as a result of the disturbance, and the pattern was similar for the two land uses (Fig. 2*b*).

In contrast to abundance, which decreased after the disturbance, species richness (rarefied) increased significantly in streams from both land uses (Fig. 2*c*; Table 2; a priori comparison before vs. after, $p < 0.023$). Eight days after the disturbance, species richness had recovered to levels similar to those of the control samples (Fig. 2*c*; Table 2; a priori comparison before vs. 8 days after, $p = 0.917$). There was no significant interaction between land use and time ($p = 0.355$; Table 2), indicating that both stream types responded similarly to the disturbance.

Fig. 4. Nonmetric multidimensional scaling (NMS) ordination of all 72 sampling units collected immediately before (solid circles), immediately after (open squares), and 8 days after the experimental disturbance (open triangles). The analysis was performed once for all samples together but was plotted separately for each stream site. For both axes, lengths are the same and were scaled to the maximum score range of the 1st axis of the analysis. Stress value is 12.2%. Sites on the left are from tussock catchments and those on the right are from pasture catchments: (*a*) Anchor, (*b*) Broad, (*c*) Clarks, (*d*) Bush, (*e*) Stone, and (*f*) M. Road.



Patchiness following the disturbance

The first axis of the exploratory NMS ordination was strongly correlated with $\log_{10}$ abundance (Fig. 4; in all six cases, $r > 0.89$, $p < 0.0001$). The second axis reflected differences in community composition and separated the two land uses only partially, producing high scores for tussock sites and low scores for pasture sites along the second axis (Fig. 4). Sampling units collected before the disturbance were scored closer to each other than those collected immediately afterwards in all three tussock sites and, to a lesser extent, in one stream draining pasture (Bush Stream). With the exception of Broad Stream, sampling units collected 8 days after the disturbance tended to be scored along the first axis at po-

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Table 3. Repeated-measures analysis of variance (ANOVA) of effects of land use (tussock vs. pasture) on mean Sørensen similarity among each of four sampling units collected at each stream site and sampling time.

	df	MS	*F*	*p*
Between subjects				
LU	1	0.0026	0.812	0.418
Streams(LU)	4	0.0032		
Within subjects				
T	2	0.0121	11.026	0.008
T × LU	2	0.0021	1.893	0.220
T × Stream(LU)	8	0.0011		

Note: *p* values for within-subjects analysis were corrected using the Huynh-Feldt method for repeated-measures ANOVA. Symbols "×" and "( )" indicate, respectively, crossing and nesting relationships among variables.

sitions intermediate to sampling units collected before and immediately after the disturbance.

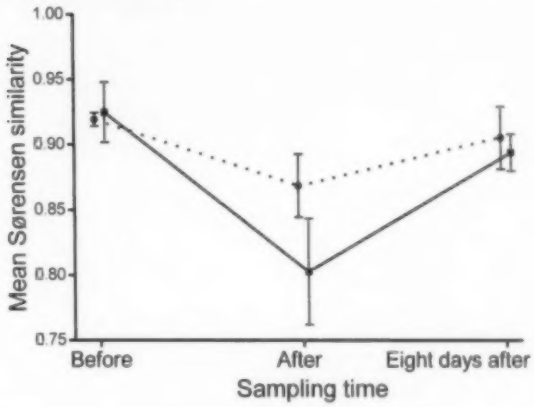
Despite the significant overall reduction in abundance detected by the ANOVA analysis, some sampling units were not affected negatively by the disturbance. Except for Bush Stream, all streams had sampling units immediately after the disturbance with similar densities to those of control sampling units (scored close to the control sampling units in Fig. 4). In two streams (Anchor and Clarks), sampling units had abundances higher than the controls (scoring to the right of the reference units in the NMS), probably reflecting the accumulation of individuals dislodged from patches affected negatively by the disturbance. We checked whether sampling units with high abundance after the disturbance were located downstream in the study sites, in which case high abundance could just be an artificial consequence of colonisation by dislodged individuals from upstream, disturbed areas. All four sampling units at each site were collected in sequence starting downstream, and for each stream, the positions of the two highest scored units were 2 and 1, 3 and 2, 2 and 3, 4 and 2, 4 and 3, and 1 and 2, respectively, for Anchor, Clarks, Stone, Broad, Bush, and M. Road. These data show that density of invertebrates after the disturbance was unrelated to location in the stream reach.

Results of the ANOVA analysis of mean Sørensen similarities were similar to those obtained using abundance (Table 3). The interaction between land use and time was not significant ($p = 0.220$; Table 3). There was no difference between land uses ($p = 0.418$), but a significant difference was found among sampling times ($p = 0.008$). Mean similarity among sampling units was high before, decreased immediately after (a priori comparison before vs. after, $p = 0.002$), and recovered to original levels by 8 days after the disturbance (a priori comparison before vs. 8 days after, $p = 0.281$; Fig. 5).

Discussion

Several experimental studies have examined the effects of disturbance on stream invertebrate communities. However, these have generally been concerned with relatively small scales, such as individual stones (Lake and Schreiber 1991),

Fig. 5. Mean Sørensen similarity in tussock (solid line) and in pasture (dotted line) stream sites for three sampling times. Data for each stream and sampling time were obtained by averaging the six pairwise comparisons among the four sampling units (Surbers). Standard errors are shown; $n = 3$ for each sampling time and catchment type.



artificial substrates (Malmqvist and Otto 1987; McCabe and Gotelli 2000), baskets filled with stones (Reice 1985; Death 1996), or small areas up to 9 m^2 (Matthaei et al. 1996, 1997). Furthermore, most of them were unreplicated (Mackay 1992; but see Death 1996). Although small-scale studies have shown some of the ways that disturbance can influence stream communities, it is likely that experiments incorporating large areas and replicated in a number of streams will produce more realistic and general results (but see discussion in Death 1996). For example, recovery in a stream patch is likely to be influenced by the surrounding patches. In studies in which the disturbance effect is applied to small patches, resilience is likely to be greater than would be observed in natural floods, where surrounding patches are generally also affected and fewer colonists are available (Brooks and Boulton 1991; but note the similarity in response of invertebrates to natural and experimental disturbance events described in Matthaei et al. (1997)).

Resistance and resilience in pasture and tussock sites

The reduction in invertebrate abundance that we found immediately after the disturbance is in accordance with previous studies of natural and experimental hydrological disturbances. We observed no changes in species density in our study, and this concurs with some previous experiments. McCabe and Gotelli (2000) listed 10 studies that evaluated disturbance effects on species density. There were reductions in species density in eight of them, whereas no change was observed in the studies of Englund (1991) and Reice (1984, 1985). In contrast to abundance, we found an increase in rarefied species richness after the disturbance event. The study of McCabe and Gotelli (2000) was the first to use rarefied species richness to measure changes in stream invertebrate diversity. They found an increase in species richness as a result of several disturbance treatments. Our experimental disturbance involved entire stream reaches, whereas those of McCabe and Gotelli (2000) were restricted to individual paving

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stones. The observation of increased species richness in two studies performed at contrasting spatial scales warrants the use of this metric in future disturbance research in streams.

Our original hypotheses that tussock streams would be more resistant and resilient in the face of a disturbance in terms of abundance, species density, and species richness was not supported, as revealed by the nonsignificance of the interaction factor in the ANOVA analyses. Compared with pasture sites, tussock sites had greater heterogeneity of substrates (coefficient of variation (CV) of particle size), which may indicate more hydraulic refuges. On the other hand, particle sizes were two to five times larger in two of the pasture streams than in tussock streams, which could confer high resistance to water flow (Downes et al. 1997). However, these habitat features were not significantly associated with higher resistance or resilience. This result partially disagrees with Death (1996), who found differences in species density, but not abundance, when comparing recovery in stable and unstable stream sites. In his study, recovery of species density in stable streams was faster than in unstable streams.

Although the lack of statistically significant differences between the land-use types in our study might represent the actual situation, we do not exclude completely the original hypothesis that assemblage structure in tussock streams would be more stable in the face of a disturbance. Our experimental disturbance was designed to mimic a natural flood. Indeed, except for one stream, abundance reductions in our study (59, 6, 51, 70, 71, and 57%, respectively, for Anchor, Clarks, Stone, Broad, Bush, and M. Road) were in the range generally found in other studies (e.g., Brooks and Boulton 1991; Matthaei et al. 1996). However, as in other experimental studies, this disturbance was not completely realistic. Although we believe that our experiment was more realistic than previous ones regarding spatial scale, we admit that it contained some differences to a real flood. Water velocities near the bed and shear stresses were probably artificially high for our chosen study streams. The water jet was able to move even the heaviest hemisphere used by Statzner and Müller (1989) to measure shear stress, indicating that shear stresses were much higher than usually recorded in floods. The experimental disturbance scoured large patches of moss in tussock sites (A.S. Melo and D.K. Niyogi, personal observations), which is unlikely to happen during real floods in these streams. An additional point is that discharge during our experimental procedure remained unchanged relative to before the experiment, perhaps preventing invertebrates from being flushed very far from the experimental area. Furthermore, rapid recovery of disturbed areas may have been in part due to drift from the undisturbed, upstream reaches (Townsend 1989).

Average resistance and, to a lesser extent, resilience were somewhat higher in tussock streams than in pasture streams, but these differences were not significant. A natural flood, large enough to cause movements of smaller stones but not detachment of moss, might have led to significant differences in resistance and resilience between pasture and tussock sites if greater substrate heterogeneity provided more refuges for invertebrates in tussock streams.

Nearly complete recovery was observed within 8 days in our study. This is in agreement with reports in the literature regarding natural spates and small-scale disturbance experiments (Brooks and Boulton 1991; Mackay 1992).

Disturbance generating patchiness

Our hypothesis that disturbance would cause a transient increase in patchiness was supported by the ANOVA analysis. Similarity among sampling units collected at a site was high before the disturbance, decreased immediately following the disturbance, and then recovered to original levels in 8 days. No differences between tussock and pasture sites were detected by the ANOVA analysis. However, the increase in patchiness following disturbance was more conspicuous for tussock than for pasture sites in the NMS analysis.

An increase in habitat heterogeneity after disturbance is predicted by the patch dynamics concept (Townsend 1989). Matthaei et al. (2000) found an increase in the number of invertebrates associated with embedded stable stones immediately after a spate and a corresponding decrease in abundance on loose, unstable stones. Similarly, spates may cause an accumulation of individuals in patches where shear stress does not increase during spates (Lancaster and Hildrew 1993). In both cases, increases in heterogeneity after the disturbance were clear, with two distinct patch types: refuges and nonrefuges. We collected all of our sampling units in similar areas in riffles; therefore, any (unmeasured) characteristic of the streambed responsible for the increase in heterogeneity is likely to be subtle.

Palmer et al. (1995, 1996) noted a redistribution of benthic copepods following disturbance, but among rather than within distinct stream habitats (sandy mid-channel, fine sediments around dams, coarse sediments around dams, and dam debris). Species found in specific habitats in the absence of disturbance were found in other habitats after a spate. As a consequence, heterogeneity among habitats was high before a disturbance (i.e., each habitat contained a specific pool of species) and tended to decrease after the disturbance. However, not all patches inside the habitats that acted as refuges (vegetation debris and fine sediments around dams) actually received individuals. Patch-specific characteristics (mainly near-bed flow and water flux) were important in determining whether or not a patch acted as a refuge during a flood.

The picture emerging from our results and previous studies is that floods cause a predictable redistribution of invertebrates. Some patches within habitats and some entire habitats lose individuals during spates, whereas some patches within habitats or entire habitats retain or receive individuals, acting as refuges.

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PERSPECTIVE / PERSPECTIVE

Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses

Igor A. Grigorovich, Robert I. Colautti, Edward L. Mills, Kristen Holeck, Albert G. Ballert, and Hugh J. MacIsaac

Abstract: Since completion of the St. Lawrence Seaway in 1959, at least 43 nonindigenous species (NIS) of animals and protists have established in the Laurentian Great Lakes, of which ~67% were attributed to discharge of ballast water from commercial ships. Twenty-three NIS were first discovered in four "hotspot" areas with a high representation of NIS, most notably the Lake Huron – Lake Erie corridor. Despite implementation of the voluntary (1989, Canada) and mandatory (1993, U.S.A.) ballast water exchange (BWE) regulations, NIS were discovered at a higher rate during the 1990s than in the preceding three decades. Here we integrate knowledge of species' invasion histories, shipping traffic patterns, and physicochemical factors that constrain species' survivorship during ballast-mediated transfer to assess the risk of future introductions to the Great Lakes. Our risk-assessment model identified 26 high-risk species that are likely to survive intercontinental transfer in ballast tanks. Of these, 10 species have already invaded the Great Lakes. An additional 37 lower-risk species, of which six have already invaded, show some but not all attributes needed for successful introduction under current BWE management. Our model indicates that the Great Lakes remain vulnerable to ship-mediated NIS invasions.

Résumé : Depuis l'ouverture de la Voie maritime du Saint-Laurent en 1959, au moins 43 espèces non indigènes (NIS) d'animaux et de protistes se sont établies dans les Grands Lacs laurentiens et la présence de ~67 % d'entre elles s'explique, croit-on, par la vidange de l'eau des ballasts des navires commerciaux. Vingt-trois des NIS ont été découvertes la première fois dans quatre « points chauds » qui abritent un grand nombre de NIS, en particulier dans le corridor du lac Huron au lac Érié. Malgré la mise en application des règlements volontaire (Canada, 1989) et obligatoire (É.-U., 1993) sur l'échange des eaux de ballasts (« ballast water exchange » (BWE)), les NIS ont été retrouvées à un taux plus élevé pendant les années 1990 que durant les trois décennies précédentes. On trouvera ici une synthèse des connaissances sur l'histoire des invasions des espèces, sur les patterns de transport maritime et sur les facteurs physicochimiques qui restreignent la survie des espèces durant leur transport dans les ballasts, ainsi qu'une évaluation des risques d'introductions futures dans les Grands Lacs. Notre modèle d'évaluation des risques identifie 26 espèces qui présentent des risques élevés et qui sont susceptibles de survivre à des transferts intercontinentaux dans les ballasts; dix de celles-ci se retrouvent déjà dans les Grands Lacs. Trente-sept espèces additionnelles, qui représentent des risques plus faibles et dont six sont déjà présentes dans les Grands Lacs, possèdent une partie seulement des caractéristiques nécessaires pour une invasion réussie dans les conditions actuelles de la gestion de BWE. Notre modèle démontre que les Grands Lacs restent vulnérables aux invasions de NIS via le transport maritime.

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I.A. Grigorovich,[1] R.I. Colautti, and H.J. MacIsaac. Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada.
E.L. Mills and K. Holeck. Department of Natural Resources, Cornell Biological Field Station, Bridgeport, NY 13030, U.S.A.
A.G. Ballert. Great Lakes Commission, 2805 South Industrial Highway, Ann Arbor, MI 48104, U.S.A.

[1]Corresponding author (e-mail: igrigorovich@mail.ru).

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Introduction

Since the settlement of North America by Europeans, human activities have facilitated introductions of nonindigenous species (NIS) into the Laurentian Great Lakes (Mills et al. 1993*a*). Thus far, some 162 aquatic species have been recognized as introduced and established in this basin (Ricciardi 2001). Discharge of ballast water by commercial ships has been identified as a major vector in the transfer of NIS to the Great Lakes over the past century (Mills et al. 1993*a*; Ricciardi 2001). Consequently, efforts to reduce the risk of future introductions have focused on ballast water management. Voluntary ballast water exchange (BWE) guidelines were initiated by the Canadian government in 1989 and made mandatory by U.S. legislation in 1993 (United States Coast Guard 1993). Vessels that carry ballast water originating from freshwater or coastal brackish-water ports outside of the Great Lakes and that declare "ballast on board" (BOB) status are subject to these regulations and are requested either to exchange ballast during their ocean voyage or to seal ballast tanks while operating on the Great Lakes (United States Coast Guard 1993). The BWE procedure is intended to purge brackish water and freshwater organisms in ballast tanks and to kill those remaining by osmotic stress (United States Coast Guard 1993).

Despite a high level of compliance with these regulations (e.g., Wiley 1995), living brackish water and freshwater species continue to arrive to the Great Lakes in vessels that report open-ocean BWE, indicating that the current ballast management strategy may not fully protect the lakes from additional invasions (Locke et al. 1993). Vessels declaring "no ballast on board" (NOBOB) status are not subject to ballast management regulations; however, they enter the Great Lakes carrying unpumpable residual ballast water and sediment that contain viable organisms (e.g., Locke et al. 1991, 1993; Bailey et al. 2003). Thus, activity of NOBOB ships may predispose the lakes to invasion risk.

Modeling ballast-mediated introductions has allowed an initial assessment of risk arising from ballast water releases from the two categories (i.e., BOB and NOBOB) of ships entering the Great Lakes (MacIsaac et al. 2002). This model predicted that NOBOB vessels apparently pose a greater collective risk of ballast-mediated introductions, largely because they dominate (~90%; Colautti et al. 2003) trade into the Great Lakes and convey live organisms or viable resting stages. Other models have sought to identify potential invaders to the Great Lakes based on species invasion histories elsewhere and inbound shipping patterns (Mills et al. 1993*b*; Ricciardi and Rasmussen 1998). These forecasts have had only limited success thus far, perhaps because the ability of NIS to survive ballast-mediated dispersal was not considered explicitly.

In this study, we conduct a retrospective analysis of the mechanisms and hypotheses associated with introductions of aquatic animals and protists into the Great Lakes since the completion of the St. Lawrence Seaway in 1959. We examine transoceanic shipping traffic in the Great Lakes during 1959–2000 to explore patterns of BWE for BOB and NOBOB ships entering this basin. Moreover, we synthesize information regarding the timing and location of first occurrence, dispersal vectors, and life history traits for NIS recorded in the Great Lakes and contrast these characteristics with patterns of shipping traffic and BWE. In addition, we develop a multistep risk-assessment approach to forecast potential invaders to the Great Lakes by integrating current knowledge of shipping traffic to the basin, species' invasion histories in potential donor regions, and life history traits that facilitate survival in ballast water.

Materials and methods

Historical and emerging patterns of introduction

We assembled a comprehensive inventory of the nonindigenous aquatic metazoans and protists recorded in the Great Lakes basin from 1959 through 1999. To appraise the emerging patterns of NIS introductions under ballast water management in this basin, we chose the mandatory BWE legislation (1993, U.S.A.). Although Wiley (1995) reported that ~90% of ships complied with the Canadian voluntary guidelines enacted in 1989, many vessels likely undertook only partial exchange (e.g., Drake et al. 2001). Both BWE regulations were considered when analysing the chronology of NIS discovery. Time trends in discovery of NIS during 1959–1988 (before the ballast regulation period) were contrasted with those during 1989–1999 (after the ballast regulation period) by comparing regression line slopes. The chronological arrangement of NIS records, however, may not fully represent the rate of invasions because of time lags between the initial inoculation of NIS and their discovery (see below).

Introduced species were defined as those that successfully spread beyond their historic or native geographic range and colonized the Great Lakes via natural or human-mediated dispersal vectors. We did not distinguish between NIS whose introductions resulted from transfer across broad geographic distances (e.g., *Schizopera borutzkyi*) and those that represented local dispersal from an adjacent area (e.g., *Cyclops strenuus*). We considered only those NIS known or suspected to be introduced to the Great Lakes since the completion of the St. Lawrence Seaway in 1959. We included *Pisidium supinum* as it was discovered shortly after the opening of this seaway, although it may have established earlier and remained undetected. Several species (e.g., *Lepisosteus platostomus*) historically occurred in close proximity to the Great Lakes basin and were found in it after 1959. Following Kolar and Lodge (2002), we considered these species as introduced. We excluded species considered indigenous to any part of the Great Lakes basin but which were subsequently introduced to other, previously uncolonized sites within the basin (e.g., *Gasterosteus aculeatus*). We focused on NIS that have been documented as present and reproducing on at least two occasions, sensu Ruiz et al. (2000). We identified 12 nonindigenous invertebrates and fishes that were reported since 1959 but not established (as above) in this basin (I. Grigorovich, unpublished data); these species are beyond the scope of this survey.

For each NIS we sought to characterize the (1) date of first appearance; (2) locality of the first observation in the basin; (3) native range before the opening of the St. Lawrence Seaway; and (4) the most plausible vector (mecha-

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nism) of introduction to the basin. Entry mechanisms were categorized as follows: (1) shipping and boating activities, including transport in liquid or solid ballast, in or on cargo, or on hulls or other components of commercial or recreational boats; (2) stocking of nontarget species resulting from aquaculture; (3) release of aquarium species; (4) bait release; (5) canal development; (6) multiple vectors involving more than one entry mechanism; in such cases, the most plausible mechanism was indicated; and (7) natural dispersal vectors (e.g., wind or birds). Parasites and epibionts (e.g., *Trypanosoma acerinae* and *Acineta nitocrae*) were assigned the same entry mechanism as that of their host species, except when they were inadvertently released through stocking activity (e.g., *Glugea hertwigi*). Where no entry mechanism was indicated in the original record of a species, we synthesized information on transfer vectors from other published and unpublished studies. We acknowledge that the inherent limitation of this approach precludes rigorous hypotheses testing.

Spatial patterns of species introductions in the Great Lakes were analyzed by mapping sites of the first observations of NIS in the basin. We selected relatively small, semi-enclosed areas, nearly surrounded by land, that encompass the highest density of sites where NIS were first discovered in the Great Lakes. Areas that contribute to the diversity of the Great Lakes' nonindigenous fauna were defined here as "invasion hotspots", featuring >1 NIS per 1000 km^2 of water surface area. Lake surface areas were measured by outputting images of sections enclosing the sites of NIS to Scion Image for Windows software, Beta 4.0.2 release, on a personal computer.

Shipping activities and ballast water discharge

We developed a comprehensive database of transoceanic ships operating on the Great Lakes during 1959–2000 using annual reports of the St. Lawrence Seaway Development Corporation (SLSDC), the U.S. Customs forms 1400 and 1401 (1981–1986), the U.S. Maritime Administration's (MARAD) reports (1987–2000), records compiled by Norm Eakins (1995, 1996, 1997, 1998, 1999, 2000, 2001), and Rene Beauchamp's unpublished data (9041 Bellerive, Montréal, QC H1L 3S5; list of sources consulted available from K. Holeck).

The principal limitation of our data set is that, for 1981–1993, we were unable to determine the ballast status of transoceanic vessels that entered U.S. ports after visiting Canadian ports because these vessels were indicated as "foreign" entrances in U.S. Customs and MARAD reports. Thus, some vessels classified as "from foreign" in these sources did not necessarily originate from overseas. Therefore, when examining the redistribution of Great Lakes' ballast water by foreign vessels, we used a subset of the data that included only those ships that proceeded to a U.S. port directly from overseas and had a clearance directly to an overseas port. From 1959 through 1979, SLSDC reports listed the combined numbers of inbound and outbound annual trips for each category (BOB and NOBOB) of transoceanic vessel. We estimated the number of inbound trips for transoceanic BOB ships by comparing numbers of inbound and outbound trips operated by transoceanic BOB ships with all inbound trips operated by transoceanic and domestic BOB ships with all inbound and outbound trips operated by all transoceanic and domestic BOB vessels. The number of inbound transits for NOBOB ships was estimated in a similar way. We inferred ballasting and deballasting operations by the type of transaction recorded at each port. For example, if cargo was unloaded, we assumed that ballasting occurred, whereas deballasting was assumed when cargo was loaded. If a ship visited several ports to load or unload cargo, we assumed that this vessel loaded or discharged ballast in proportion to the number of ports it visited. We assumed that ballast water was discharged at or near the port at which cargo was loaded, except when ships "lighten the load" by releasing some water before entering shallow passages and connecting channels (e.g., St. Clair, Detroit, and St. Mary's rivers and the Welland Canal). We ascribed ballast releases in connecting waterways to the immediate downstream lake, except the Detroit River, which was, together with the St. Clair River, ascribed to Lake St. Clair.

Forecasting introductions

Recent developments in invasion biology provide the basis for predictive risk assessment of NIS (e.g., Carlton 1996; Kolar and Lodge 2001, 2002). We developed a qualitative risk-screening framework by integrating species characteristics and factors that are important at different stages of ballast-mediated introduction. These stages (i.e., uptake, transportation, release, establishment, and spread) represent sequential transitions that NIS must complete in the invasion process (Fig. 1).

We first analyzed the geographic origin of vessels entering the Great Lakes using information on port of origin, Great Lakes' port of arrival, and whether ships carried ballast water (i.e., BOB status) or cargo (i.e., NOBOB status) while inbound (Eakins 1998; C. Major, St. Lawrence Seaway Management Corporation, Cornwall, Ont., personal communication). For the purpose of our analysis, each port of origin was assigned to broad oceanic and continental regions (as below; see Donor regions). We identified the major donor regions from whence >50% of transoceanic vessels inbound to the Great Lakes originated during 1997. We were unable to obtain similar comprehensive data for other years.

For these donor regions, we assembled a list of nonindigenous aquatic invertebrates and protists with documented invasion histories. In addition, for the Black Sea basin, we considered indigenous species that were introduced to previously uncolonized sites within this and two adjacent (Caspian and Azov seas) basins via human activities. Although our approach can be extended to other taxonomic groups (e.g., fishes), these were not considered here because of insufficient evidence of their commensalism with ballast water transport vectors in donor regions. However, Kolar and Lodge (2002) developed a predictive risk assessment for nonindigenous fishes.

From the list of potential invaders, we identified species capable of completing their life cycle entirely in Great Lakes' milieu using water temperature and salinity characteristics of their native habitats. Inferences were drawn from the published literature, unpublished reports, dissertations, and our own field observations (I. Grigorovich, unpublished data). We excluded species that are unlikely to pass through the climatic and salinity screening.

Next we judged whether species could exploit ballast water transport vectors by examining evidence for their occur-

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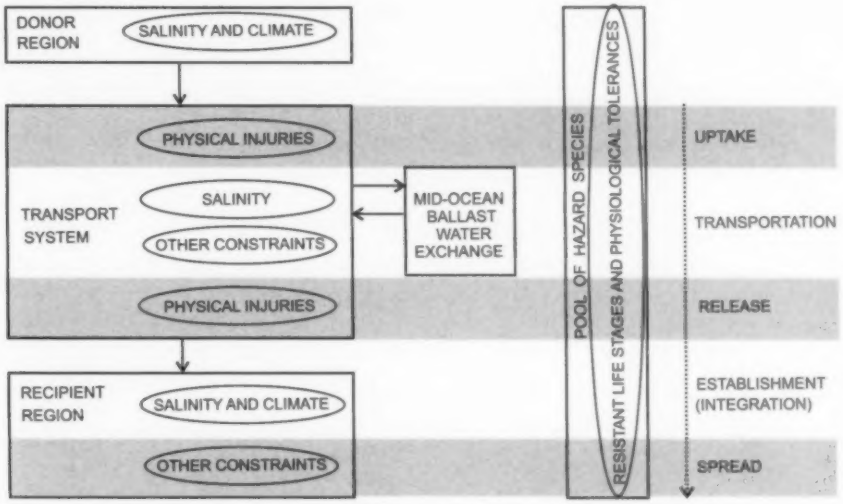
Fig. 1. Conceptual model of ballast-mediated introduction (modified from Carlton (1985)). (Left) Donor and recipient ecosystems interact via ships carrying ballast water and associated biota. (Right) Ballast-mediated introduction process compartmentalized into sequential transitions that invading species must complete to proceed in invasion. Corresponding events on both sides are linked with shaded and blank areas. Number of propagules is predicted to decline with each subsequent transition (MacIsaac et al. 2002). Sampling of ballast tanks revealed that 80–100% of coastal organisms loaded aboard were eliminated during transoceanic transit by open-ocean ballast exchange (Wonham et al. 2001).



rence in ballast medium from ballast tank surveys. For those taxa that are likely to be transported in ships' ballast water but not recorded aboard vessels, we required evidence of unintentional secondary spread along invasion corridors (Ricciardi and MacIsaac 2000).

Then we assessed which species, at any life stage, are likely to survive the adverse conditions associated with ballast-mediated transfer. Information on species characteristics was collected from published literature, unpublished reports, and dissertations (I. Grigorovich, unpublished data). Invasion risk was assessed for (*i*) BOB ships that conduct open-ocean BWE by examining species' tolerances to salinities of 17‰ and (*ii*) NOBOB ships by examining species' ability to produce resistant life stages and (or) tolerate reduced oxygen content. Other environmental factors that may impair the physiological condition and survivorship of inoculants during transport in NOBOB ballast tanks (e.g., food limitation) were not considered because they have been studied inadequately (see Wonham et al. (2001) for list of factors). Finally, we estimated the capability of species to become established. Species that possess life history traits and environmental tolerances amenable to transportation and survival in ballast tanks were categorized as posing a high risk for establishment in the Great Lakes. Species that passed through all but the last screen were considered as posing a lower risk of invasion.

Retrospective analysis of NIS in the Great Lakes

We identified 43 aquatic animal and protist species as introduced and established in the Great Lakes basin since 1959 (Table 1). Ballast water transport by commercial vessels was invoked to explain the origin of 67% of these NIS (Fig. 2). In addition, two species, the copepod *Skistodiaptomus pallidus* and bivalve *Corbicula fluminea*, likely gained entry via boating activities not associated with ballast water. Although ballast water discharge is a very likely mechanism by which many NIS have been introduced to the Great Lakes, only a few taxa have been collected from ballast tanks: the copepod *Cyclops strenuus*, cladoceran *Bosmina coregoni*, and unidentified veligers of the bivalve *Dreissena* (Howarth 1981; Locke et al. 1993; Aquatic Sciences 1996). In most cases, possible involvement of alternative vectors was rarely examined owing to a dearth of data, and evidence of entry mechanisms was inferred after the discovery of new NIS (see Daniels (2001) for examples). For example, several entry mechanisms, including stocking, river drainage divergence, canals, ballast water release, and bait release, operating individually or jointly, may account for the introduction of *Cyclops strenuus* (Smith and Fernando 1978; Reed and McIntyre 1995; Aquatic Sciences 1996).

Even though shipping is considered the only apparent vector for four fishes and their parasites introduced since 1959 (Table 1), none of these fishes has ever been collected in ballast water (e.g., Wonham et al. 2000). Stocking of salmonid fishes from Atlantic and Pacific drainages, Europe, or other parts of the world may have served as the entry mechanism for some parasites in the Great Lakes (e.g., Crafford 2001). Furthermore, no live fish have been detected in residual water of 38 NOBOB ships sampled during 2001–2002 (S. Bailey and C. van Overdijk, Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, unpublished data). Possible reasons include the following: small apertures (typically 1–2 cm) of intake covers precluding uptake of large-bodied animals; ability of fish to avoid

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Table 1. Nonindigenous metazoans and protozoans recorded in the Great Lakes basin since 1959, arranged chronologically.

No.	Taxonomic group	Species name	Year of discovery	Native region	Entry vector	Reference
1	Mollusca	*Pisidium supinum*	1959	Europe	B	Mackie 1999
2	Protista	*Glugea hertwigi* *	1960	Eurasia	S	Dechtiar 1965; Mills et al. 1993*a*
3	Pisces	*Lepisosteus platostomus*	1962	Mississippi basin	C	Fuller et al. 1999; Cudmore-Vokey and Crossman 2000
4	Crustacea	*Bosmina coregoni*	1966	Eurasia	B	Wells 1970; Deevey and Deevey 1971; Mills et al. 1993*a*
5	Crustacea	*Skistodiaptomus pallidus*	1967	Mississippi basin	B	Patalas 1969; Robertson and Gannon 1981; Mills et al. 1993*a*
6	Platyhelminthes	*Dugesia polychroa*	1968	Europe	B	Ball 1969
7	Protista	*Myxosoma cerebralis* §	1968	Unknown	M (S)	Anonymous 1988; Hoffman and Schubert 1984
8	Pisces	*Enneacanthus gloriosus*	1971	Atlantic North America	M (A)	Werner 1972; Mills et al. 1993*a*
9	Crustacea	*Cyclops strenuus*	1972	Boreal Holarctic	N ?	Selgeby 1975; Reed and McIntyre 1995
10	Crustacea	*Nitocra hibernica*	1973	Eurasia	B	Czaika 1978; Hudson et al. 1998
11	Pisces	*Notropis buchanani*	1979	Mississippi basin	M (F)	Holm and Coker 1981; Mills et al. 1993*a*
12	Mollusca	*Corbicula fluminea*	1980	Asia	M (B)	Clarke 1981; McMahon 2000
13	Annelida	*Ripistes parasita*	1980	Eurasia	B	Barton and Griffiths 1984
14	Pisces	*Alosa aestivalis*	1981	Atlantic North America	M (C)	MacNeill 1998
15	Crustacea	*Bythotrephes longimanus*	1982	Eurasia	B	Bur et al. 1986; O. Johannsson and W. Taylor, personal communication[a]
16	Annelida	*Gianius aquaedulcis*	1983	Europe	B	Farara and Ersues 1991
17	Crustacea	*Salmincola lotae* §§	1985	Eurasia	?	Lasee et al. 1988
18	Pisces	*Apeltes quadracus*	1986	Atlantic North America	M (B)	Holm and Hamilton 1988
19	Pisces	*Gymnocephalus cernuus*	1986	Eurasia	B	Simon and Vondruska 1991
20	Crustacea	*Bosmina maritima*	1988	Europe	B	De Melo and Hebert 1994
21	Crustacea	*Argulus japonicus* †	1988	Asia	A	Galarowicz and Cochran 1991; Mills et al. 1993*a*
22	Mollusca	*Dreissena polymorpha*	1988	Ponto-Caspian	B	Hebert et al. 1989
23	Mollusca	*Dreissena bugensis*	1989	Ponto-Caspian	B	May and Marsden 1992; Mills et al. 1999
24	Pisces	*Alosa chrysochloris*	1989	Hudson and Mississippi basins	C	Fago 1993; Fuller et al. 1999
25	Pisces	*Neogobius melanostomus*	1990	Ponto-Caspian	B	Jude et al. 1992
26	Pisces	*Proterorhinus marmoratus*	1990	Ponto-Caspian	B	Jude et al. 1992
27	Mollusca	*Potamopyrgus antipodarum*	1991	Australasia	B	Zaranko et al. 1997
28	Crustacea	*Onychocamptus mohammed*	1992	Holarctic	B ?	Hudson et al. 1998; P. Hudson, personal communication[b]
29	Platyhelminthes	*Dactylogyrus amphibothrium* ‡	1992	Eurasia	B	Pronin et al. 1998
30	Platyhelminthes	*Dactylogyrus hemiaphibothrium* ‡	1992	Eurasia	B	Pronin et al. 1998
31	Platyhelminthes	*Neascus brevicaudatus* ‡	1992	Eurasia	B	Pronin et al. 1998
32	Platyhelminthes	*Ichthyocotylurus pileatus* ∫	1992	Black Sea basin	B	Pronin et al. 1997
33	Protista	*Trypanosoma acerinae* ‡	1992	Eurasia	B	Pronin et al. 1998
34	Crustacea	*Echinogammarus ischnus*	1994	Ponto-Caspian	B	Witt et al. 1997; van Overdijk et al. 2003

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Table 1 (*concluded*).

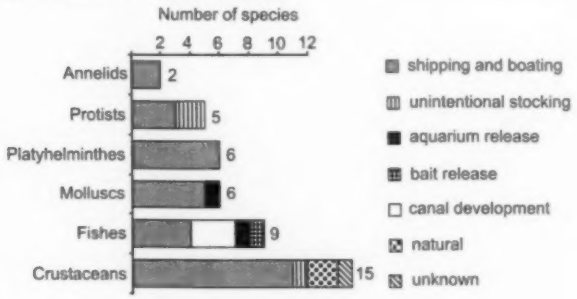
No.	Taxonomic group	Species name	Year of discovery	Native region	Entry vector	Reference
35	Crustacea	*Neoergasilus japonicus* ††	1994	Eastern Asia	M (S)	Hudson and Bowen 2002
36	Protista	*Sphaeromyxa sevastopoli* ∫ ‡‡	1994	Black Sea basin	B	Pronin et al. 1997
37	Platyhelminthes	*Scolex pleuronectis* ∫	1994	Black Sea basin	B	Pronin et al. 1997
38	Crustacea	*Heteropsyllus* cf. *nunni*	1996	Eurasia	B	Horvath et al. 2001
39	Protista	*Acineta nitocrae* +	1997	Europe	B	Grigorovich et al. 2001
40	Crustacea	*Cercopagis pengoi*	1998	Ponto-Caspian	B	MacIsaac et al. 1999
41	Crustacea	*Schizopera borutzkyi*	1998	Ponto-Caspian	B	Horvath et al. 2001
42	Crustacea	*Nitocra incerta*	1999	Ponto-Caspian	B	Grigorovich et al. 2001
43	Crustacea	*Daphnia lumholtzi*	1999	Australasia/Africa	N ?	Muzinic 2000; I. Grigorovich, unpublished data

Note: Keys to vectors of introduction: B, boating including recreational and ballast-mediated vectors; C, canal development; A, aquarium release; S, stocking of nontarget species with aquaculture; F, bait release; M, multiple vectors; N, natural vectors; ?, unknown or uncertain. In case of possible multiple mechanisms, the most plausible vector was indicated in parentheses. Parasite and epibiont hosts: *, *Osmerus mordax*; §, *Tubifex tubifex* and salmonid spp.; §§, *Lota lota*; †, *Carassius auratus*; ‡, *Gymnocephalus cernuus*; ∫, *Neogobius melanostomus*; ††, cyprinid, percid, and centrarchid spp.; ‡‡, *Proterorhinus marmoratus*; +, *Nitocra* spp.

[a]O. Johannsson, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Department of Fisheries and Oceans, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

[b]P. Hudson, Great Lakes Science Center, United States Geological Survey, 1451 Green Road, Ann Arbor, MI 48104, U.S.A.

Fig. 2. Taxonomic diversity of aquatic nonindigenous metazoans and protozoans established in the Laurentian Great Lakes since 1959, sorted by entry mechanism.



sampling efforts in ballast tanks; or inhospitable environmental conditions precluding fish survival (e.g., Wonham et al. 2000).

Ballast water and sediment may provide an excellent avenue for dispersal of copepods and some branchiopods because they avoid unfavourable conditions by entering diapause or by producing resting stages (e.g., Horvath et al. 2001). Hudson et al. (1998) suggested such an entry mechanism for the copepod *Megacyclops viridis*. However, its broad Holarctic occurrences, including recent findings in the Canard River drainage, western Lake Erie basin, indicate that *M. viridis* is naturally distributed in the Great Lakes (Monchenko 1974; I. Grigorovich, unpublished data). Another copepod, *Acanthocyclops americanus*, has been collected from ships entering the Great Lakes and identified as a potential invader (Locke et al. 1993; MacIsaac 1999); but it was earlier regarded as native to Lake Michigan based on historical and biogeographical evidence (e.g., Monchenko 1974). Thus, based on circumstantial evidence, shipping has been inferred to explain the origin of these species, though, in fact, they could be Great Lakes' natives.

Several taxa that are poorly differentiated morphologically (e.g., sphaeriid bivalves and oligochaetes) may include cryptic invaders that were introduced before 1959 but have remained undetected for an extended time. These species include the oligochaetes *Stylodrilus heringianus*, *Spirosperma ferox*, *Potamothrix* spp., and *Branchiura sowerbyi* and the sphaeriid bivalve *Pisidium moitessierianum* (Cook and Johnson 1974; Grigorovich et al. 2000; Spencer and Hudson 2003). Some of these (e.g., *P. moitessierianum*) are not widely distributed in the Great Lakes basin, despite possessing a broad inland distribution in Europe (Grigorovich et al. 2003).

Below we analyze the temporal and spatial patterns of shipping activities in the Great Lakes to explore the involvement of ballast water transport vectors in the transfer of NIS.

Patterns of shipping activities and ballast water discharge

The overall pattern evident in transoceanic shipping traffic into the Great Lakes since 1959 is that over 90% of foreign vessels entered this system carrying cargo (i.e., NOBOB status). Only in the years 1980 and 1982 did BOB vessels outnumber NOBOB ships. The volume of transoceanic vessel traffic varied tremendously from 1959 through 2000 (Fig. 3*a*). After the opening of the St. Lawrence Seaway, traffic increased, peaking at >1430 vessels in 1967. Then traffic declined to its lowest point in 1991 (362 vessels) but increased thereafter to 675 vessels in 2000. The fraction of ballasted ships has diminished since 1992. The volume of cargo transported into the Great Lakes changed continually over the past four decades, cycling from low (e.g., 1.5 million tonnes (t) in 1961) to high (e.g., 8.6 million t in 1998) periods (Fig. 3*b*). Tonnage has increased since 1993, when the mandatory BWE legislation became effective (Fig. 3*b*). For example, average cargo volume during 1959–1992 and 1993–2000 was 4.8 and 7.0 million t·year^{-1}, respectively.

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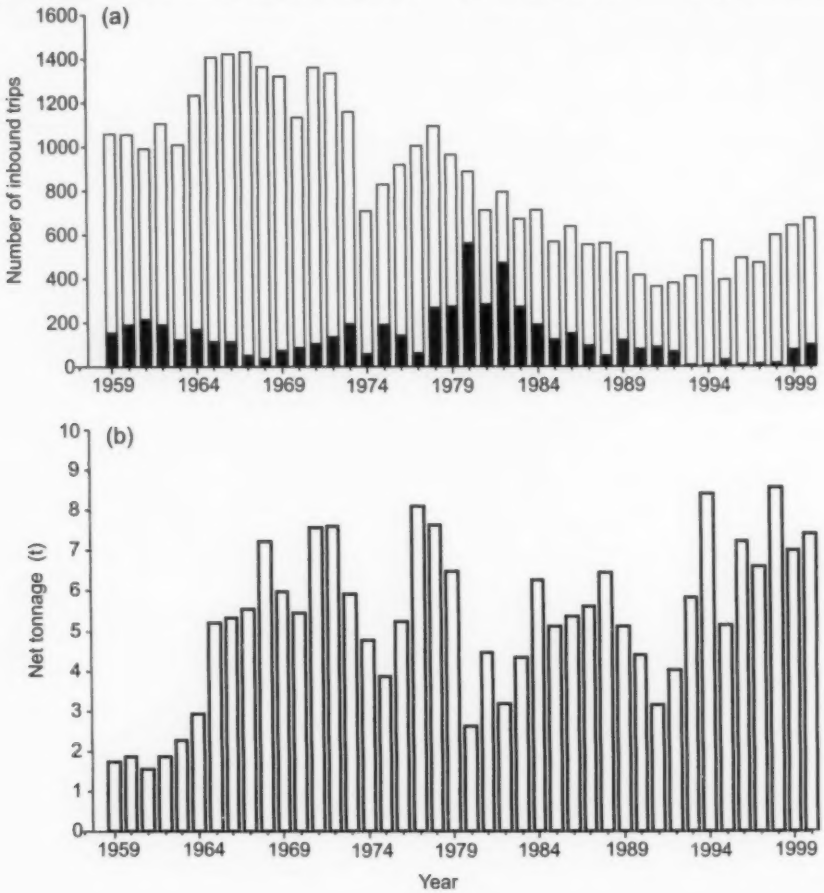
Fig. 4. Shipping activities of transoceanic vessels in the Great Lakes from 1981 through 2000: Lake Superior (solid bars), Lake Michigan (bars with grid), Lake Huron (bars with diagonal hatching), Lake Erie (shaded bars), Lake St. Clair (open bars), and Lake Ontario (bars with vertical lines). Numbers above bars represent inbound trips to United States' ports directly from overseas, based on traceable records from 1981 through 2000. (*a*) Percentage of ballast water discharges by ballasted ships into each lake. (*b*) Percentage of first port-of-call visitations by vessels carrying cargo (NOBOB status) into each lake. (*c*) Percentage of ballast water discharges by NOBOB ships into each lake.

Fig. 3. Shipping activities of transoceanic vessels in the Great Lakes from 1959 through 2000: (*a*) inbound trips of ballasted ships (solid bars) and those that declare "no ballast on board" (NOBOB) status (open bars); (*b*) net tonnage (t) of NOBOB ships.



During 1981–2000, most (~75%) BOB vessels entering the Great Lakes proceeded directly to Lake Superior, where almost all of these ships discharged their ballast load. Other recipients of ballast water from BOB ships, in order of decreasing supply, were Lake Erie, Lake Michigan, and Lake St. Clair (Fig. 4*a*).

During 1981–2000, ~72% of NOBOB vessels made their first stop at Lake Erie, where they typically unloaded cargo and loaded Great Lakes' water (Fig. 4*b*). Ports on Lake Superior received ~70% of the mixed ballast water discharges from NOBOB vessels, followed, in turn, by Lake Erie (16%), Lake Michigan (12%), and Lake St. Clair (2%) (Fig. 4*c*).

Thus, over the past four decades, the bulk of transoceanic vessels entered the Great Lakes carrying cargo and having no declarable ballast water on board. After unloading freight at Great Lakes' ports, these ships typically filled their ballast tanks with Great Lakes' water to compensate for loss of cargo weight. As a result, residual ballast water and sediment became mixed with Great Lakes' water and, after the subsequent discharging within the system, could facilitate invasions (Bailey et al. 2003). Below we examine the chronology and locations of Great Lakes' NIS discoveries to deduce their relationship to the ballast water discharge by overseas vessels.

Emerging invasion patterns

We identified four invasion "hotspots": the Lake Huron – Lake Erie corridor (10 species), Lake Erie – Lake Ontario

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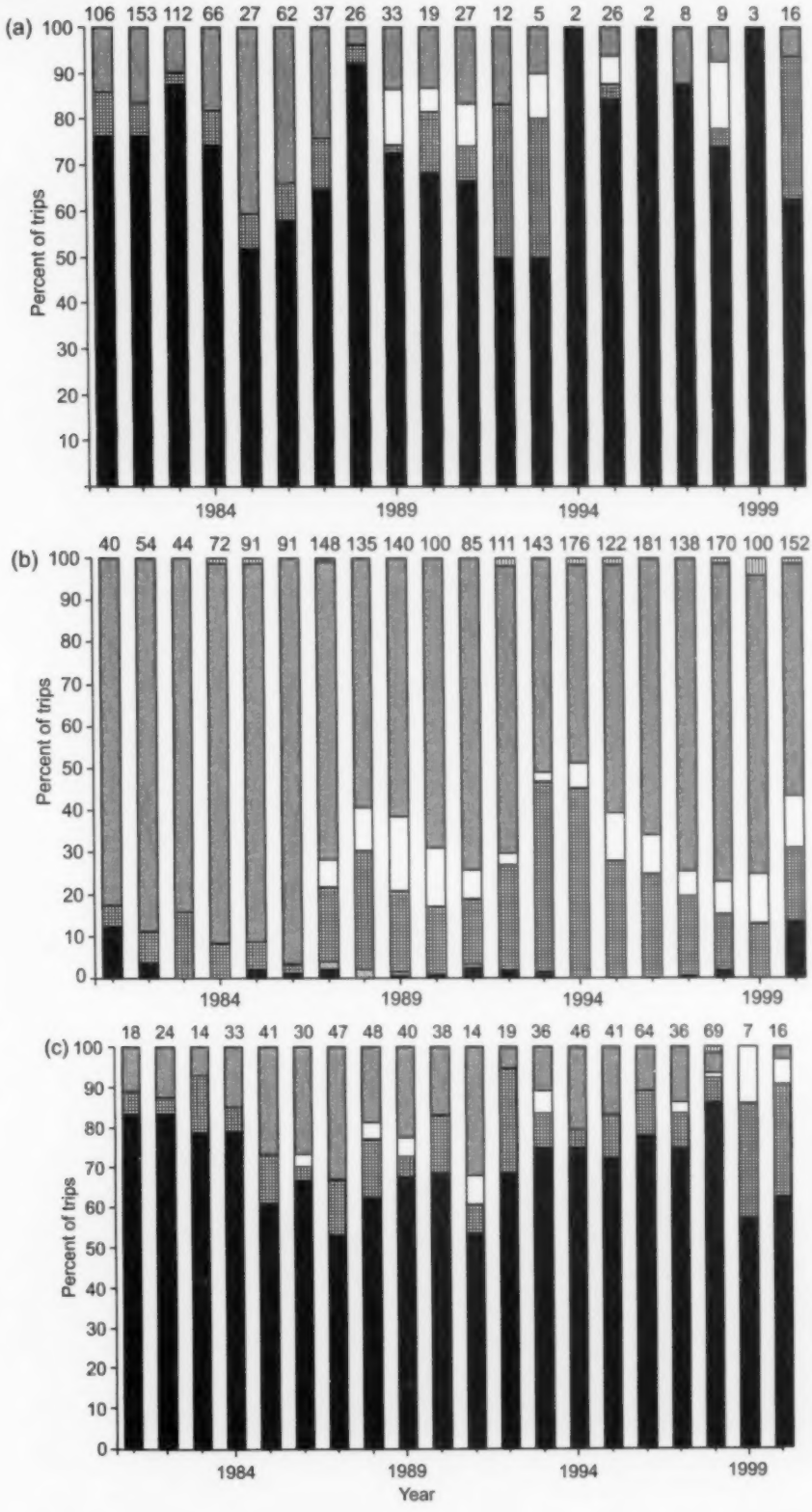

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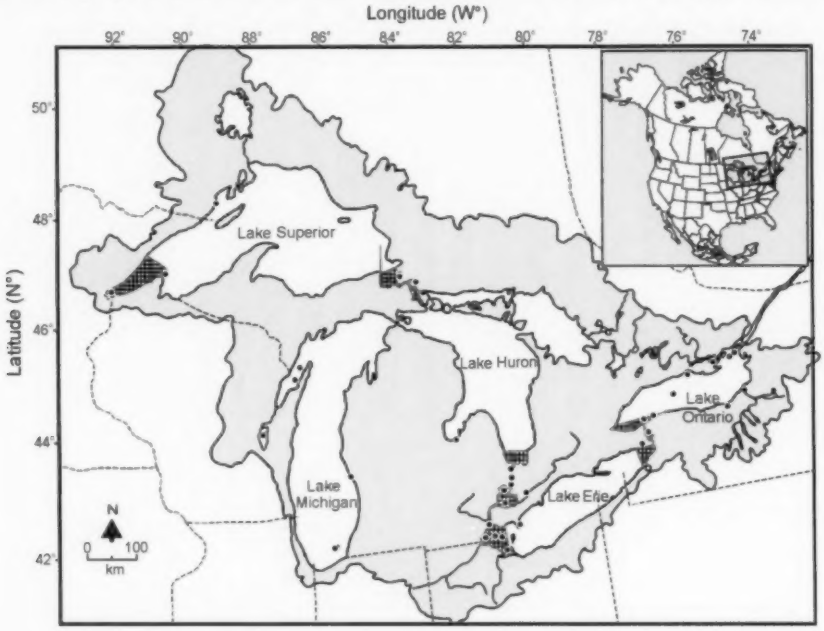
Fig. 5. Map of the Laurentian Great Lakes drainage (shaded) showing sites of the first discovery for established nonindigenous aquatic animals and protists (●) 1959–1999. Fish hatchery location, where *Myxosoma cerebralis* was first discovered in North America, is indicated by ○. Invasion "hotspots" featuring high concentrations of new nonindigenous species are indicated by hatching of lake surface area.



corridor (4 species); Lake Superior – Lake Huron corridor (2 species); and the western end of Lake Superior (7 species) (Fig. 5). Collectively these hotspots compose <5.6% of the total Great Lakes' water surface area but account for ~53.5% of NIS recorded since 1959. Thus, the concentration of NIS sites in these hotspots is more than 19 times that in the remaining lake surface area. The density of NIS sites ranges from >2/1000 km^2 in the Lake Huron – Lake Erie corridor to ~1/1000 km^2 in the Lake Superior – Lake Huron corridor. At a large spatial scale, these hotspots generally correspond to the major areas of ballast water discharge by ocean-going vessels, including western Lake Superior, the Lake Huron – Lake Erie corridor, and the Lake Erie – Lake Ontario corridor (Aquatic Sciences 1996). Also, these hotspots are all fairly shallow water areas. It is possible that before entering the shallow passages, ships "lighten up" by discharging some ballast water to reduce draft, in accordance with safety operational requirements (Carlton et al. 1995). Some of the NIS findings in the interconnecting channels could be explained by drift from upstream locations (e.g., western Lake Erie from the Detroit River). Further research is necessary to determine why NIS are concentrated in hotspot areas.

Although the "propagule pressure" hypothesis attributes invasion outcome to the quantity of NIS propagules delivered, formal records of ballast water discharge are a poor predictor of the extent of ballast-mediated invasions in the Great Lakes (Grigorovich et al. 2003). For example, despite receiving a disproportionate ballast water and presumably propagule supply, the western end of Lake Superior supports relatively few (~14%) NIS (species 17, 19, and 29–33 in Table 1). Five of these NIS (species 29–33) were likely transported with their host (*Gymnocephalus cernuus*) and, as such, may represent a single inoculation event. A number of hypotheses may account for the relative rarity of NIS in this lake (see Grigorovich et al. 2003).

The rate of discovery of nonindigenous invertebrates and protists in the Great Lakes has changed dramatically through time (Fig. 6*a*). The number of new NIS recorded between 1989 and 1999 increased to three times the rate observed between 1959 and 1988 (Fig. 6*b*). This pattern may be real, reflecting changes in vector type and magnitude between these periods. Alternatively, this pattern may be an artifact of sampling bias, reflecting increasing awareness of, and sampling efforts for, NIS (e.g., Ricciardi 2001). Between 1959 and 1999, there was no consistent relationship between the number of new NIS discovered in the Great Lakes and volume of shipping activities per 5-year period (Fig. 6*b*). Some NIS (e.g., *Daphnia lumholtzi*) are associated with vectors other than shipping. Inherent time lags between the initial inoculation of NIS and their identification in the field may also partially account for the "accelerating" accumulation of introduced species during the 1980s and 1990s. Records of deliberate introductions indicate that successfully introduced populations may remain undetected for an extended time following initial stocking, from 1 year (Karpevich 1975) to 20 years (reviewed in Grigorovich et al. 2002). Likewise, time lags between the initial inoculation and detection are anticipated for inadvertently introduced NIS (Sakai et al. 2001). However, no data exist allowing for an accurate estimation of the duration of time lags in the Great Lakes. The problem of time lags may be particularly acute when new invaders

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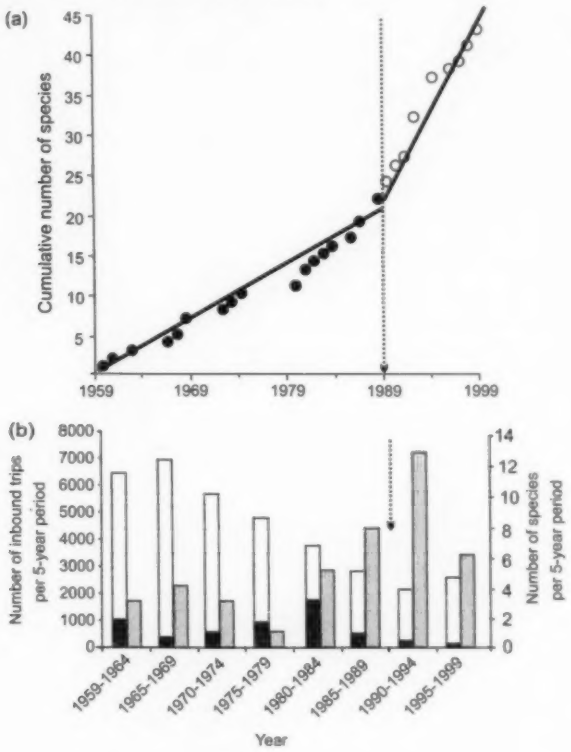
Fig. 6. Time trends in introductions of nonindigenous metazoans and protozoans in the Great Lakes from 1959 through 1999. (*a*) Cumulative number of introduced species recorded over the preregulation period (1959–1988; slope = 0.621, r^2_{adj} = 0.963) and after ballast regulation period (1989–1999; slope = 1.880, r^2_{adj} = 0.956). The species accumulation rate during the period before ballast water regulation (0.621) lies outside the 95% confidence (1.543–2.216) interval of the postregulation period. (*b*) Number of species discovered (shaded bars) and volume of inbound traffic by overseas ballasted (solid bars) and cargo-carrying ships (open bars) per 5-year period. Implementation of the voluntary guidelines for the control of ballast water discharges (1989) is indicted by arrows.



are not demonstrably distinct in morphology from taxa already resident in the system. For example, the time lag extended over 100 years for the morphologically cryptic sphaeriid bivalve, *Pisidium moitessierianum*, established in the Great Lakes (Grigorovich et al. 2000). Only 10 NIS (~23%) in our survey (species 8, 12, 13, 15, 19, 22, 25, 26, 40, and 43 in Table 1) are deemed clearly distinctive morphologically from species resident in the system, whereas the remaining NIS may have been subject to time lags.

The ecological profile of nonindigenous fauna introduced through ships' ballast water has changed over the period of 1959–1999. Nonindigenous species inhabiting the water–sediment interface have become predominant since 1993 (Table 1). This change may reflect a shift in transport vectors from those associated with BOB vessels to those associated with NOBOB vessels (see Fig. 2*a*). Six NIS species discovered after 1993 (species 38–43 in Table 1), excluding parasites of previously established fishes, may have been transported to the Great Lakes in ballast tanks of NOBOB ships. An additional species, *Echinogammarus ischnus*, was present in western Lake Erie in 1994 and may have been introduced as early as 1993 (van Overdijk et al. 2003).

Forecasting ballast-mediated introductions

Our risk-assessment framework predicts potential invertebrate invaders and estimates the risk of their successful introduction in the Great Lakes using a sequence of "screens" (after Hayes 1998; Haugom et al. 2002). Each screen may independently preclude invasion or establishment of NIS (Fig. 7). These screens include the following: (1) strong shipping pathways and vector activities linking high-risk donor regions with the Great Lakes; (2) history of invasiveness and commensalism with humans; (3) salinity and climate matches between recipient and donor regions; (4) interface with ballast water vectors and availability for ballast intakes; and (5) life histories and environmental tolerances that may enhance inoculant survival during uptake and transportation in ballast tanks.

Donor regions

From 1983 through 1998, trade into the Great Lakes was dominated by vessels arriving from European ports (~88%), followed by those originating from South and Central America. In total, we identified 460 different last ports-of-call, representing virtually all regions of the world, from whence Great Lakes shipping traffic originated. In 1997, five regions accounted for >72% of Great Lakes' inbound traffic, namely the North Sea, the Baltic Sea, the South American Atlantic, the North American Atlantic, and Black Sea basins (Fig. 8). Of these, basins of the North, Baltic, and Black seas contributed ~55% of inbound vessels. Although a high proportion of overseas vessels do not operate on regular, defined routes (Niimi 2000), the former two basins remained the primary sources of Great Lakes' shipping traffic during 1983–1998, whereas vessel traffic from the Black Sea basin was difficult to discern but clearly was much lower than that from the former two regions (Colautti et al. 2003). The North American Atlantic region was not considered here because it is a source of cabotage trade. The South American Atlantic region was excluded because of insufficient information on the extent of NIS introductions in this region and because of its pronounced climatic mismatch with the Great Lakes region (as below) precluding establishment of propagules. Thus, some species posing a risk of invasion cannot be predicted by our model because certain ports of ballast origin were not considered.

Invasion history

We identified 73 and 52 nonindigenous invertebrates that have documented invasion histories in the North Sea and Baltic Sea basins, respectively (Gollasch and Leppäkoski 1999; Kelleher et al. 1999; Van der Velde et al. 1999; Hopkins 2001; Bij de Vaate et al. 2002). We also identified 168 invertebrate species that have been deliberately or accidentally introduced in the Ponto-Caspian basins and thus are likely to

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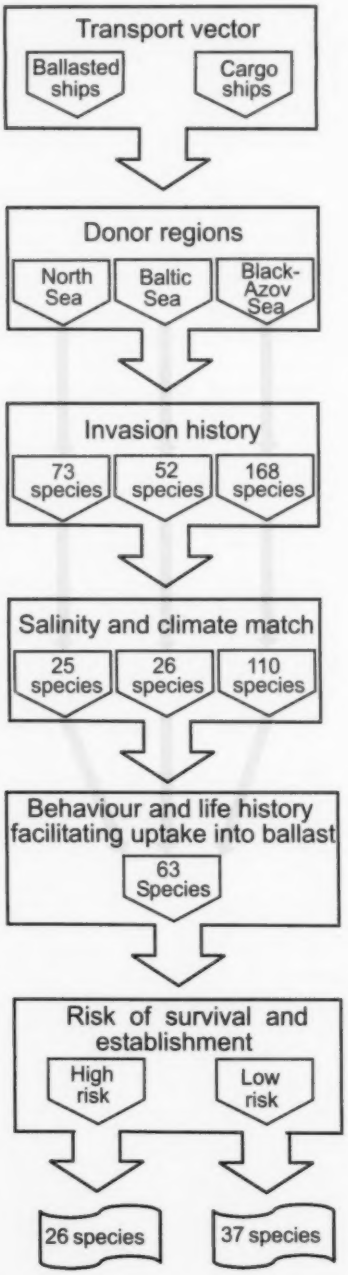
Fig. 7. Risk-assessment framework for prediction of ballast-mediated introductions into the Laurentian Great Lakes. Risk-assessment screens may independently or jointly preclude transfer, survival, or establishment of species in the invasion process. Screening of target species originating from individual donor regions is indicated by shading.



interface with relevant vectors for transfer of aquatic species elsewhere (Grigorovich et al. 2002; see Grigorovich et al. (2003) for updated list of nonindigenous and cryptogenic metazoans and protists in the Great Lakes). Twenty-seven species, including 16 Ponto-Caspian natives, have documented invasion histories in either the Baltic Sea or North Sea basins (Gollasch and Leppäkoski 1999; MacIsaac et al. 2001; Bij de Vaate et al. 2002). Putative sources of recent NIS in the Great Lakes are generally consistent with these three regions (Ricciardi and MacIsaac 2000; MacIsaac et al. 2001). Thus, we identified a total of 238 species with histories of introductions in the areas from whence most Great Lakes' shipping traffic originates.

Salinity and climate matches

We used water temperature and salinity parameters of their native habitats to determine whether species could become established. For example, water temperature was judged to deter the gastropod *Melanoides tuberculata* from establishment as it needs minimum temperatures of ~18°C (Duggan 2002). Temperature changes in ballast tanks could further decrease the probability of its survival during transit to the Great Lakes.

We excluded species that were judged unlikely to survive or reproduce in fresh water. For the latter reason, the Chinese mitten crab, *Eriocheir sinensis*, has failed to establish self-sustaining populations, even though it has been repeatedly transported to the Great Lakes (Nepszy and Leach 1973; Council of Great Lakes Fishery Agencies 2001). Likewise, the Atlantic–Mediterranean peracarid crustaceans *Gammarus aequicauda* and *Idotea baltica*, which are capable of surviving at salinity as low as ~0.1‰, were rejected because they require a mixohaline environment (1–30‰) for reproduction (Karpevich 1975).

The resulting 139 species, including euryhaline forms and freshwater specialists, were judged likely to pass through the climate screening as their potential source regions — the coasts of the North, Baltic, and Black seas — are climatically compatible with the Great Lakes region (e.g., Reid and Orlova 2002).

Shipping vector interface

Organisms occurring in the water column in sufficient quantities, including holoplankton, meroplankton, and nekton, were judged likely to be loaded with ballast water (following Carlton 1985; Carlton and Geller 1993; Haugom et al. 2002). We excluded fish parasites (e.g., parasitic helminthes of Amur cyprinid fishes) for which establishment relies on the introduction of their host. We screened out aquatic organisms with behavioural and life history features that make them unavailable for ballast water intake, including sessile taxa with short planktonic stages and absent dormant life stages (e.g., bryozoan *Urnatella gracilis*) and molluscs that burrow in sediment (e.g., brooding sphaeriids, *Pisidium* spp.). In addition, we rejected large-bodied crayfishes (e.g., *Astacus leptodactylus*) because they may be precluded from uptake by intake hatch screens and because they are unlikely to be present in the water column during the ballasting phase.

Sediment stirring in shallow ballasting ports may enable benthic organisms to be drawn into ballast intake streams (e.g., Gollasch and Leppäkoski 1999). Therefore, we also included free-living nektonic (e.g., gammarid amphipods) and benthic organisms, including those dwelling in attached tubes but which migrate occasionally (e.g., corophiid amphipods,

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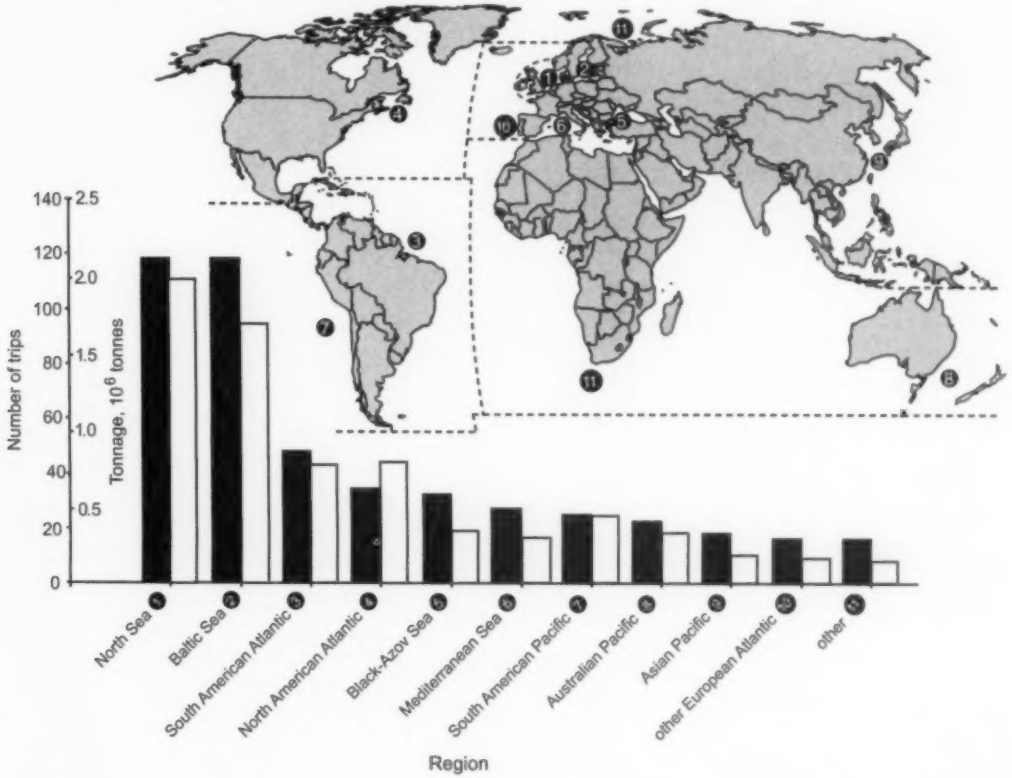
Fig. 8. Source regions of cargo-carrying ships (NOBOB) inbound to the Great Lakes in 1997. Shipping activity is represented by number of inbound trips (open bars) and by net tonnage (solid bars). In addition to 444 inbound trips operated by NOBOB vessels in 1997, Eakins (1998) reported 31 trips by ballasted vessels.



ampharetid polychaetes). Analysis of the transfer mechanisms for these species revealed that dispersal beyond their natural range may have involved shipping or multiple, interacting transport vectors (Grigorovich et al. 2002).

A total of 63 species likely pass through the ballasting phase of the ballast water transfer sequence. These species represent a variety of life styles (plankton, benthos, and nekton), life history strategies (*r*- and *K*-selected strategies), and salinity tolerances (freshwater–oligohaline, mesohaline, euryhaline).

Ballast tank survival

We estimated the likelihood of potential survival of target species for NOBOB and BOB vessels. Using salinities of species' natural habitats, we first gauged the risk of propagule survival in ballasted vessels that comply with BWE legislation. We considered coastal and estuarine species that have evolved osmoregulatory mechanisms allowing for rapid ion exchange regulation when moving between salt water and freshwater habitats (e.g., Mordukhai-Boltovskoi 1960, 1970). Species that naturally occur at salinities of 17‰ (e.g., amphipods *Echinogammarus warpachowskyi* and *Dikerogammarus villosus*; Derzhavin 1951; Bruijs et al. 2001) were judged to survive a partial to complete BWE, provided that they have not been flushed out from ballast tanks in the process. We considered eight species to have a high probability of surviving ballast-mediated transfer and establishment (Table 2). Of these, the littoral gammarid amphipod *Pontogammarus maeoticus* is likely to be taken on board in small quantities, deterring its establishment. Therefore, we considered it as a low-risk species. The remaining target species were not considered for BOB ships, as they are not known to survive at high salinities (as above). Also, we did not assess risk level for dormant life stages of freshwater species in BOB ships, because limited data suggest that high salinity water may reduce their viability (Bailey et al. 2003).

When assessing the risk of propagule survival in NOBOB vessels, we considered various invertebrate taxa that tolerate some exposure to anoxia, feed on detritus or sediment, and show burrowing behaviours. These taxa were judged likely to survive in NOBOB ballast tanks as evidenced from sampling residual ballast water and sediment (Gollasch and Leppäkoski 1999; S. Bailey, Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, unpublished data). Six species (13–18 in Table 2) were judged to have a high likelihood of surviving ballast-mediated transfer because they may enter diapause as adults and thereby survive anoxic conditions in ballast tanks.

Benthic organisms that lack resistant pelagic life stages or behaviours (e.g., gastropods except *Potamopyrgus antipodarum* assessed for risk above) were judged likely to have low density in residual ballast water resulting from the fewer

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Table 2. List of species predicted to invade the Great Lakes using risk-based model of ballast-mediated introduction.

No.	Risk level	Taxonomic group	Species name	Donor region	Ship category
1	High	Coelenterata	*Cordylophora caspia* *+	N, B, P	BOB
2		Crustacea	*Daphnia cristata* ‡	P	BOB, NOBOB
3			*Bosmina coregoni* *‡	P	BOB, NOBOB
4			*Bosmina obtusirostris* ‡	P	BOB, NOBOB
5			*Bythotrephes longimanus* *‡	N, B, P	BOB, NOBOB
6			*Cercopagis pengoi* *‡	B, P	BOB, NOBOB
7			*Cornigerius maeoticus maeoticus* ‡	P	BOB, NOBOB
8			*Podonevadne trigona ovum* ‡	P	BOB, NOBOB
9			*Eurytemora affinis* *‡	P	BOB, NOBOB
10			*Heterocope appendiculata* ‡	P	NOBOB
11			*Heterocope caspia* ‡	P	NOBOB
12			*Calanipeda aquae-dulcis* ‡	P	BOB, NOBOB
13			*Cyclops kolensis* ‡	P	NOBOB
14			*Ectinosoma abrau*	P	NOBOB
15			*Paraleptastacus spinicaudata triseta*	P	NOBOB
16			*Schizopera borutzkyi* *	P	NOBOB
17			*Nitocra incerta* *	P	NOBOB
18			*Onychocamptus mohammed* *	P	NOBOB
19			*Hemimysis anomala* +	N, B, P	BOB, NOBOB
20			*Echinogammarus ischnus* *+	N, B, P	BOB, NOBOB
21			*Echinogammarus warpachowskyi* +	P	BOB, NOBOB
22			*Dikerogammarus villosus* +	N, P	BOB, NOBOB
23			*Dikerogammarus haemobaphes* +	N, P	BOB, NOBOB
24			*Pontogammarus aralensis* +	P	BOB, NOBOB
25			*Pontogammarus robustoides* +	B, P	BOB, NOBOB
26		Mollusca	*Potamopyrgus antipodarum* *+	N, B, P	BOB, NOBOB
27	Low	Platyhelminthes	*Dendrocoelum romanodanubiale*	N	NOBOB
28			*Apophallus muehlingi*	P	NOBOB
29			*Nicolla skrjabini*	P	NOBOB
30			*Rossicotrema donicum*	P	NOBOB
31		Annelida	*Hypania invalida*	N, P	NOBOB
32			*Hypaniola kowalevskyi*	P	NOBOB
33			*Potamothrix bedoti* *	B	NOBOB
34			*Potamothrix heuscheri*	B	NOBOB
35			*Potamothrix moldaviensis* *	B	NOBOB
36			*Potamothrix vejdovskyi* *	B	NOBOB
37		Crustacea	*Paramysis lacustris*	B, P	NOBOB
38			*Paramysis intermedia*	P	NOBOB
39			*Limnomysis benedeni*	N, B, P	NOBOB
40			*Pterocuma pectinata*	P	NOBOB
41			*Pseudocuma cercaroides*	P	NOBOB
42			*Proasellus coxalis*	N	BOB, NOBOB
43			*Proasellus meridianus*	N	BOB, NOBOB
44			*Jaera sarsi*	P	NOBOB
45			*Jaera istri*	N	NOBOB
46			*Gammarus tigrinus*	N, B	BOB, NOBOB
47			*Gmelinoides fasciatus*	B, P	NOBOB
48			*Pontogammarus maeoticus* +	P	BOB, NOBOB
49			*Pontogammarus subnudus*	P	NOBOB
50			*Pontogammarus crassus*	P	NOBOB
51			*Pontogammarus obesus*	P	NOBOB
52			*Echinogammarus berilloni*	N	NOBOB
53			*Echinogammarus trichiatus*	N, P	NOBOB
54			*Iphigenella shablensis*	P	NOBOB
55			*Corophium curvispinum*	P	NOBOB
56		Mollusca	*Theodoxus fluviatilis*	P	NOBOB

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Table 2 (*concluded*).

No.	Risk level	Taxonomic group	Species name	Donor region	Ship category
57			*Theodoxus pallasi*	B, P	NOBOB
58			*Lithoglyphus naticoides*	N, B, P	NOBOB
59			*Corbicula fluminea* *	N	NOBOB
60			*Corbula gibba*	N	NOBOB
61			*Dreissena polymorpha* *	N, B, P	NOBOB
62			*Dreissena bugensis* *	P	NOBOB
63			*Hypanis colorata*	P	NOBOB

Note: Species occurring at salinities of 17‰ are indicated by +. Species that have already established in the Great Lakes are marked with *. Species producing resistant life stages are indicated by ‡. Keys to donor regions: N, North Sea basin (lower Rhine River); B, Baltic Sea basin; P, Black Sea – Azov Sea basin. Invasion risk was assessed for ballasted (BOB) and those that declare "no ballast on board" (NOBOB) status.

propagules loaded during the ballasting phase or poor survivorship during transit. Consequently, they were considered to pose a lower risk of establishment. Obligate sexual reproduction may further deter these taxa from becoming established owing to Allee effects (species 37–55 in Table 2). For example, the Ponto-Caspian amphipod *Corophium mucronatum* was found, but has not established, in Lake St. Clair, perhaps because of demographic limitations (Grigorovich and MacIsaac 1999). Consequently, these organisms were considered to pose a lower risk of establishment (see Table 2 for list of species).

In addition, species that are known to produce resistant life stages (e.g., ephippia, resting eggs, cysts), and thereby survive transfer in residual ballast water or sediments in NOBOB vessels, were judged likely to pose a relatively high risk of introduction and establishment. Bailey et al. (2003) have experimentally assessed the viability of resting stages for rotifer, cladoceran, and calanoid copepod species in ballast tanks of NOBOB ships.

Predicted and actual invertebrate invaders

Our model identified 63 species that pose an invasion risk to the Great Lakes. Of these, 26 species are considered high-risk invaders, including 10 species that invaded the lakes between 1959 and 1999 (Table 2). These NIS may have been transported directly from the Black Sea basin (species 1, 3, 5, 6, 9, 16–18, 20, and 26 in Table 2) or indirectly from the North Sea (species 5, 20, and 26) or Baltic Sea (species 1, 6, 20, 26) basins, which may serve as "distribution hubs" (sensu Ricciardi and Rasmussen 1998). The remaining 16 high-risk species have not yet been reported in the Great Lakes. This group consists of four cladoceran, six copepod, and six peracarid crustaceans. Five peracarid crustaceans (species 19, 21–23, and 25 in Table 2) native to the Ponto-Caspian basins have established in the Rhine River or Baltic Sea basins (e.g., MacIsaac et al. 2001). Euryhaline adaptations of these species, in conjunction with their establishment in staging areas that are linked to the Great Lakes via "invasion corridors", may facilitate their transfer in ships' ballast tanks to the Great Lakes (e.g., Bruijs et al. 2001). Each cladoceran and copepod species from this group possesses at least two features that render them suitable to a ballast water vector: (1) ability to enter diapause as an adult; (2) production of dormant life stages; (3) single-parent reproduction; or (4) short generation times. The ubiquity of these features in NIS established since 1993 may have fostered survival of adverse conditions associated with ballast-mediated transfer and ensured rapid population growth in novel habitats.

An additional group of 37 species is considered to pose a lower risk of invasion to the Great Lakes under the mandatory BWE legislation. Of these, three species of tubificid oligochaetes (species 33, 35, and 36 in Table 2) and three species of bivalves (species 59, 61, and 62) established before implementation of BWE regulations. Five of these species are Ponto-Caspian natives, and one, *Corbicula fluminea*, is native to Southeast Asia. The oligochaetes have an invasion history in the Baltic Sea and may have gained entry to the Great Lakes in solid ballast before the early 1900s (see Mills et al. 1993*a*). Possible geographical sources of the zebra mussel, *Dreissena polymorpha*, include the regions of the Black, North, or Baltic seas, whereas the quagga mussel, *Dreissena bugensis*, could originate only from the Ponto-Caspian basins (Mills et al. 1999; Grigorovich et al. 2002; Vanderploeg et al. 2002).

A remaining 31 species pose a lower risk of invasion and have not yet been found in the Great Lakes. These species typically lack physiological tolerances or life history traits that enhance survival during unfavourable conditions encountered during ballast water transfer. This group includes 19 peracarid crustacean, four platyhelminth, five mollusc, and three annelid species. These species may be introduced directly from their native habitats or indirectly from invaded Baltic Sea and North Sea habitats. NOBOB vessels may facilitate these introductions, as they are exempt from BWE regulations.

In conclusion, we identified 43 nonindigenous animal and protist species established in the Great Lakes basin since 1959. More than 53% of these NIS were first recorded in four invasion hotspots where ballast water is likely discharged from overseas vessels. The overall rate of invasion of NIS increased dramatically in the 1990s, although several factors may account for this pattern. Using species invasion histories, shipping traffic to the Great Lakes, species characteristics, and factors critical to invasion success, we predict that an additional 47 invertebrate species pose a hazard of invasion. We identify 16 species that are capable of surviving ballast-mediated transport and pose a high risk for establishment in the Great Lakes. Additionally, 31 species are considered to pose a lower risk of invasion. The shift in shipping from BOB to NOBOB vessels during the 1980s may have served as a filter that favoured invasion of species capable of diapause or production of resistant life stages. This change

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may signal a new phase in the history of anthropogenic transformations of the Great Lakes' biota.

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CORRIGENDUM / RECTIFICATIF

Corrigendum: Opposite effects of ocean temperature on survival rates of 120 stocks of Pacific salmon (*Oncorhynchus* spp.) in northern and southern areas

Franz J. Mueter, Randall M. Peterman, and Brian J. Pyper

Ref: Can. J. Fish. Aquat. Sci. **59**: 456–463 (2002).

In Table 1 on p. 460, parameter estimates for southern sockeye salmon (*Oncorhynchus nerka*, last row) were estimated based on effective female spawners rather than total spawners, which were used for all other stocks. Hence the productivity estimate (α) is biased high and other parameter estimates may be biased as well. Corrected parameter estimates for sockeye stocks in area "south" are as follows: average stock productivity (α) = 1.87; first-order autocorrelation (ϕ) = 0.186; regional sea surface temperature (SST) effects: $\gamma = -0.145^{**}$, $SE_\gamma = 0.048$, $\Delta R = -12\%$; and large-scale Pacific Decadal Oscillation (PDO) effect: $\gamma = -0.101^{**}$, $SE_\gamma = 0.034$, $\Delta R = -8\%$ (**denotes significance at $P < 0.01$).

In Fig. 2, also on p. 460, the estimated random SST effects for sockeye salmon in the south now have a smaller estimated variance as a result of refitting the model. This implies a narrower spread of the random effects (solid triangles below horizontal line in Fig. 2*c*) and a more peaked appearance of the corresponding estimated probability density function (Fig. 2*f*).

In Fig. 1 on p. 458, axes were labeled incorrectly. The *x*-axis label should read "Longitude (°W)" and the *y*-axis label should read "Latitude (°N)".

The Acknowledgements should have included the following statement: "COADS data provided by the NOAA–CIRES Climate Diagnostics Center, Boulder, Colo., U.S.A., from their web site at http://www.cdc.noaa.gov/".

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F.J. Mueter,[1,2] R.M. Peterman, and B.J. Pyper.[3] School of Resource and Environmental Management, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada.

[1]Corresponding author (e-mail: fmueter@alaska.net).
[2]Present address: 697 Fordham Drive, Fairbanks, AK 99709, U.S.A.
[3]Present address: Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 11120 Glacier Highway, Juneau, AK 99801, U.S.A.

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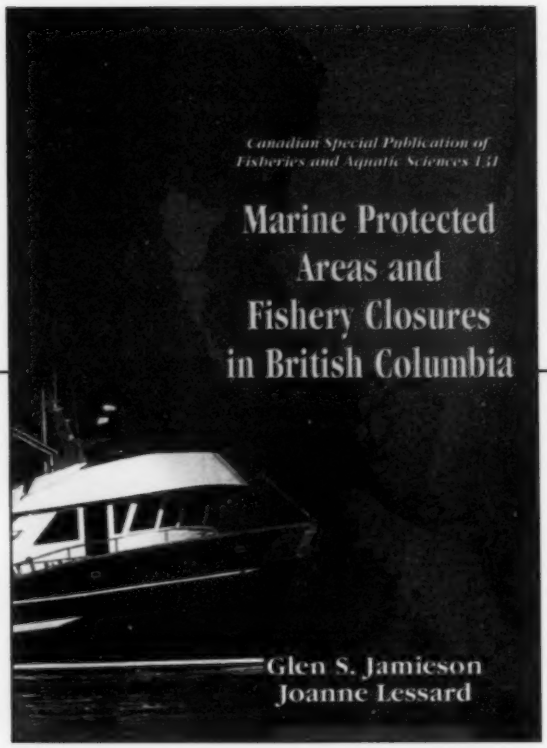
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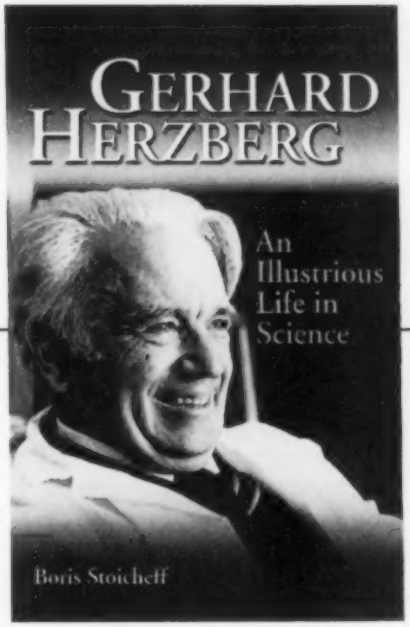

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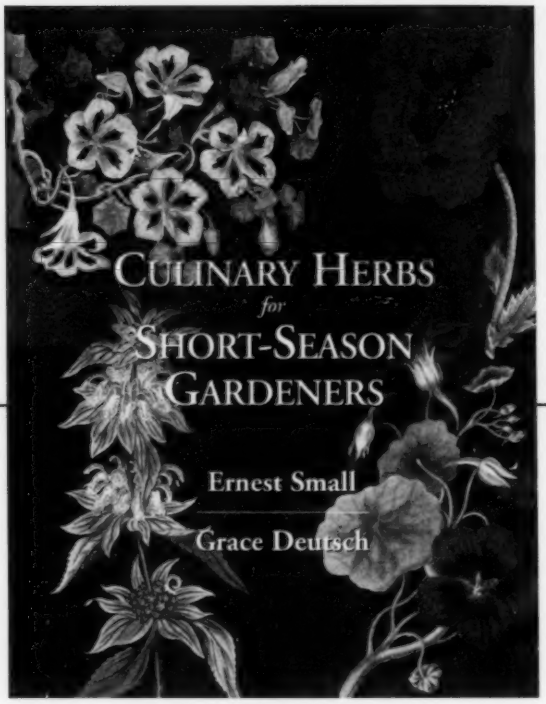

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